1984

Characteristics Of Human Cytotoxic Lymphocytes Specific For Haptenic And Non-major Histocompatibility Complex (mhc) Cell Surface Antigens

Michael John Ward

CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

CHARACTERISTICS OF HUMAN CYTOTOXIC LYMPHOCYTES' SPECIFIC FOR HAPTENIC AND NON-MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CELL SURFACE ANTIGENS

by

Michael John Ward

Department of Microbiology and Immunology

Faculty of Medicine

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Canada

November, 1983

ABSTRACT

Cell mediated immune responses (CMIR), mediated by thymus derived (T) lymphocytes, have been shown to play a central role in specific tumour immunity, autoimmune disease, and allograft rejection. Studies of specific cytotoxic responses indicate that the cells involved in the CMIR are capable of recognizing various cell surface markers including histocompatibility antigens, chemically modified cell surface antigens and viral antigens on the cell surface. Although many investigations have been conducted the exact nature of the target antigens and the role of the recognitive ability of the responding cells during clinically related problems, such as autoimmune disease and allograft rejection, are not known.

Sensitization of human T cells in Marbrook tissue culture vessels and microtitre trays resulted in the production of effector cells capable of lysing certain ^{51}Cr labelled target cells. The characterization of the reactivity of these cells was carried out in two systems, the first in which the target cell had been chemically modified with trinitrobenzene-sulphonic acid, and the second in which the target cells were either obtained from healthy, normal volunteer donors or from donor-recipient transplant pairs.

The effector cell capable of lysing chemically modified targets did not exhibit MHC restricted cytotoxicity. The

cell was a large granular lymphocyte that resided in the non-T cell fraction of cells separated through sheep erythrocyte rosette formation. The cytotoxicity could also be developed, without specific sensitizing antigen, in the presence of factors derived during the generation of an allogeneic response. On the basis of the above observations it was concluded that the predominant effector cell within this system could be a human natural killer (NK) cell.

Cytotoxic lymphocytes capable of lysing unmodified cells were directed at either major histocompatibility complex (MHC) coded targets or at stable, non-MHC coded targets. Reactivity against the non-MHC coded target antigens was specific but included a form of reactivity that exhibited a broad range of lysis. Three types of reactivity were delineated; 1) MHC explainable, 2) explainable on the basis of specific antigens on the cell surface that are not MHC antigens, and 3) reactivity that is not specific for a particular cell surface antigen but is due to target susceptibility and/or effector reactivity. This last form of cytotoxicity may be based on the genetic makeup of the particular cells involved, on the mode of preparation and handling of the cells, or the assay conditions.

Analysis of the distribution of reactivity within the two systems suggest that the primary CMIR is directed against modified self components of the target cell and that it is mediated by an NK-like cell. This form of reactivity is observed between MHC identical transplant pairs, certain

randomly selected individuals from the normal population, and in response to hapten and tumour antigen altered self. Greater antigenic differences activate more specific T cells that mediate a selective, MHC specific response that is the major form of reactivity observed in anti-allogeneic responses or during allograft rejection.

ACKNOWLEDGEMENTS

I would like to thank the following people who, through their various contributions, made this work possible:

Dr. N. R. StC. Sinclair for his patience and thoughtful discussion through the years.

D. McGirr for her valuable input and enthusiasm that instilled in me the necessity of always controlling for the unexpected.

The late M. Fung for his knowledge and patience in teaching me the methods and techniques used throughout the thesis.

Dr. W. T. Howson and the members of the transplant laboratory team for support and discussion.

D. McFarlane for his time spent helping me master STA-PUT velocity sedimentation techniques and his assistance during the antibody titrations.

Dr. A. M. Jevnikar for many invaluable discussion periods spent in the wee hours of the morning over cups of coffee and microtitre trays.

Finally I would like to thank all the agencies that supported this work and hope that they will never underestimate the need for basic research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

C'	Complement
CD	Cellular defined
CML	Cell mediated lympholysis
CMIR	Cell mediated immune response
DR	D region associated
E	Erythrocyte
$F(ab')_2$	Fragment antigen binding, divalent
Fc	Fragment crystallizable
FCS	Fetal calf serum
GvH	Graft vs host
HBSS	Hank's balanced salt solution
HIR	Humoral immune response
HLA	Human leukocyte antigen
H-2	Histocompatibility-2 (mouse MHC)
Ia	Immune region associated
Il-2	Interleukin-2
KLH	Keyhole limpit haemocyanin
LD	Lymphocyte defined
Ly	Lymphocyte antigen
MLC	Mixed lymphocyte culture
MLR	Mixed lymphocyte reaction
MHC	Major histocompatibility complex
NK	Natural killer cell
OKT	Human T cell antigen series
PBL	Peripheral blood lymphocytes
PBS	Phosphate buffered saline
PHA	Phytohaemagglutinin
RBC	Red blood cells
RM	Rosetting medium
s	Sedimentation coefficient
SERF	Sheep erythrocyte rosette forming
SD	Serologically defined
SRBC	Sheep red blood cells
TC	Tissue culture
$T\gamma$	T cells with IgG receptors
$T\mu$	T cells with IgM receptors
TNBS	Trinitrobenzenesulphonate
TNP	Trinitrophenol
V region	Variable region

This work is dedicated to my parents who helped me when
I really needed it, and left me alone the rest
of the time

GENERAL REVIEW

1. Introduction

The ability of the immune response to discern self from non-self is one of the most important factors determining the survival of complex multicellular organisms. The critical function of immunological surveillance (Burnet, 1970) is responsible for maintaining the integrity of an organism against both exogenous and endogenous influences and is carried out primarily by two distinct sets of antigen reactive cells. The first cell type, known as B cells, arises in the Bursa of Fabricius in birds (Warner et al., 1962; Cooper et al., 1965) and the bone marrow in man (Stutman and Good, 1969) before settling at various sites in the peripheral lymphoid tissues. Once at these sites they give rise to antibody secreting cells upon activation with antigen (Claman and Chaperon, 1969; Davies, 1969; Miller and Mitchell, 1969). The second cell type migrates from the bone marrow to the thymus where it acquires certain functional properties. These thymus derived or T lymphocytes are responsible for the various phenomena of cell mediated immunity (Claman and Chaperon, 1969; Davies, 1969; Miller and Mitchell, 1969).

Studies of the immune system in both invertebrate and vertebrate species suggest that the cell mediated immune

vertebrate species suggest that the cell mediated immune response (CMIR) evolved prior to the humoral or antibody mediated immune reponse (HIR) (Burke, 1970; Gatti et al., 1970). Invertebrate defense mechanisms generally appear to be non-specific and consist primarily of phagocytosis and intracellular digestion (Davis et al, 1973), although specific destruction of integumental allografts in earthworms has been reported (Cooper, 1965). The lamprey, a member of the order Cyclostomata, is among the most primitive of the lower marine vertebrates and possess a primitive, non-encapsulated thymus as well as circulating lymphoid cells. Although lymphocytes exhibiting obvious morphological differences are present, no members of the plasma cell series have been demonstrated (Good et al., 1966). The elasmobranchs (cartilaginous fish) have a well developed encapsulated thymus and can evoke an antibody mediated response against some antigens (Finstad and Good, 1966). Bony fish, reptiles, and amphibians have a more highly developed thymus, concommitant with increased immunological responsiveness and the production of antibodies sedimenting in the 7s and 19s fractions (Good et al., 1966). Secondary lymphoid organs responsible for the maturation of the HIR appear in birds (Warner et al., 1962; Cooper et al., 1965) but only mammals have true lymph nodes (Good et al., 1966). There has been evidence to suggest that non-thymus derived lymphocytes (Henny et al., 1972; Perlmann et al., 1972) and non-lymphoid cells such as

macrophages (Evans and Alexander, 1972; Lohmann-Matthes et al., 1972) can effect target cell lysis under specific *in vitro* conditions but immune T lymphocytes play the central role in cell mediated responses in higher organisms.

Landsteiner and Chase (1942) first demonstrated the ability of the immune system to elicit a CMIR by the transfer of delayed type hypersensitivity, via immune lymphocytes, to non-reactive animals. The involvment of T cells in resistance to certain microorganisms (Mackaness and Blanden, 1967), specific tumour immunity (Klein et al., 1960; Stulting and Berke, 1973), autoimmune disease (Flax and Caulfield, 1963), and allograft rejection (Lawrence, 1957) led to intensive studies in these areas, particularly with respect to the recognition of different cell surface antigens.

Immune lymphocytes sensitized *in vivo* against allografts were capable of lysing donor cells in dogs (Govaerts, 1960), mice (Rosenau and Moon, 1961), and rats (Wilson, 1963) in the absence of complement and humoral antibodies. *In vitro* generated CMIR resulted in the development of a specific cytotoxic response against allografts in mice (Golstein et al., 1972) and humans (Solliday and Bach, 1970; Sondel et al., 1975). T lymphocytes were shown to be responsive to various cell surface markers including histocompatibility antigens (Ceppellini et al., 1969; Goulmy et al., 1977), chemically modified surface antigens (Shearer et al., 1976a), and viral

antigens on the cell surface (Collins et al., 1980).

The cytotoxic capacity of a T cell directed against either virus infected or chemically modified target cells is restricted by the major histocompatibility complex (MHC) background on which these antigens are presented (Shearer et al., 1976b; Zinkernagel and Doherty, 1974a). The target cell employed in the final assay for T cell cytolytic activity must share some MHC genes with the original stimulating cell used in the induction of the response. This requirement may be the result of the antigenic determinants recognized by the cytolytic T lymphocyte (CTL) and suggests that CTL recognized altered-self antigens, thereby playing an important role in immunological surveillance (Zinkernagel and Doherty, 1974b). There have been recent reports showing a high degree of cross-reactivity between allelic variants of self-MHC and autologous MHC antigens that interact with foreign viral (Finberg et al., 1978) or chemical antigens (Burakoff et al., 1976; Shaw et al., 1978). The exact nature of the target antigen recognized by the CTL is still not clearly defined.

2. Effector T Cells

Effector T lymphocytes can be defined as the cells that are involved in the development of cytotoxic potential in response to stimulation by a foreign antigen. Regulatory T cells, which can either help or suppress a response, may be said to express an effector function by virtue of their involvement in generating an efferent response, therefore the term effector T lymphocyte can encompass those cells mediating the cytolytic activity as well as those cells contributing indirectly to the CMIR either through help or suppression. The direct involvement of lymphocytes of the T cell lineage has been confirmed for tumour immunity and allograft rejection in both mice and humans (Brunner et al., 1970; Burton et al., 1977; Sondel et al., 1975).

Cerottini et al. (1970) were the first to demonstrate directly that sensitized T cells mediated a cytotoxic response to alloantigens during in vivo graft vs host (GvH) reactions. The transfer of allogeneic spleen cells into lethally irradiated mice resulted in the production of both cytotoxic lymphocytes and alloantibodies, whereas the transfer of thymus cells yielded cytotoxic lymphocytes but no alloantibody plaque forming cells. The cytotoxic response was abrogated following treatment with anti-theta serum and complement (C') while the HIR was not affected. This antibody detected a cell surface marker found on mouse thymocytes and peripheral T cells, but not on any other

lymphoid cells, enabling researchers to easily distinguish thymus derived cells involved in an immune response. Wagner et al. (1972a) showed that the cytotoxic activity of in vitro sensitized lymphoid cells could also be abolished by treatment with anti-theta serum plus C'.

T cells have discernible heterogeneity with respect to cell surface markers and effector cell function (Cantor and Gershon, 1979). Although the theta antigen allows the separation of T cells from non-T cells, it was not until the Ly (lymphocyte) antigen specificities were defined in mice that specific functional roles could be assigned to subsets of lymphoid cells. Eight Ly specificities have been classified and, of these, four are restricted to T cells while the others are found predominantly on B cells. The first specificities were discovered by Stuck et al. (1964), subsequently characterized by Boyse et al. (1968) and called Ly1 and Ly2. The remaining T lymphocyte restricted Ly specificities Ly3 and Ly5, were identified by Boyse et al. (1970) and Komuro et al. (1975) respectively.

Cantor and Boyse (1975a) found that only 6-8% of T lymphocytes bore the $Ly23^+$ antigen marker and the effector activity resided in this cell subset. Thirty-three percent of the cells had the $Ly1^+$ phenotype which represented a helper cell fraction that amplified the response of the $Ly23^+$ effector cells (Cantor and Boyse, 1975b). The $Ly1^+$ cells recognized the immune region associated (Ia) alloantigens while the $Ly23^+$ could not (Cantor and Boyse,

1975b).

Delineation of T cell subsets through the use of cell surface markers has also been carried out in the rat (Loop et al., 1980) and humans (Schlossman et al., 1980). In humans the various subpopulations of T cells can be identified by either monoclonal antibodies (Reinherz et al., 1980) or the presence of Fc receptors for immunoglobulins (Moretta et al., 1976). Sixty to seventy percent of T lymphocytes in human peripheral blood have Fc receptors for IgM antibodies (Tμ) and twenty percent have Fc receptors for IgG (Tγ) (Reinherz et al., 1980). These populations are distinct and do not normally overlap, although switches from one phenotype to another have been reported. During an in vitro MLC response the percentage of Tμ cells decreased progressively as a function of time while the percentage of Tγ cells increased (Moretta et al., 1981). Incubation of Tγ cells with IgG immune complexes resulted in a decrease in Tγ cell numbers and a concommitant increase in the numbers of the Tμ cell population (Pichler and Broder, 1981). Both populations react similarly to the mitogen concanavalin-A but have different dose response curves when stimulated with phytohaemagglutinin (PHA) (Moretta et al., 1976).

Monoclonal antibodies directed at the OKT T cell antigen series have enabled investigators to classify human T cell subpopulations just as the Ly antigen system allowed classification in the mouse. Anti-OKT3 antibody reacts with 100% of peripheral T cells, and 95% of sheep erythrocyte

rosetting (E^+) cells, but with no other lymphoid element (Reinherz et al., 1980). Twenty percent of the population is OKT135/8$^+$ and contain the cytotoxic/suppressor T cell populations (Schlossman, 1980). Helper cells make up 60% of the population and are OKT5/8$^-$ (Reinherz et al., 1980) but OKT134$^+$ (Schlossman, 1980). All the OKT4$^+$ and OKT5$^+$ bear the Fc receptors for IgM. Tγ cells may represent a classification of cells that are active in tumour immunity, called natural killer (NK) cells, since they do not react well with anti-OKT3 antibody but do react with OKM1 monoclonal antibody (Reinherz et al., 1980). The OKM1$^+$ phenotype is found exclusively on monocytes and granulocytes. The non-T cell nature of the Tγ cell has been disputed by some investigators using highly purified E-rosetting cell suspensions, in which the proportion of Fcγ receptor positive cells was greater than the number of OKT3$^-$ cells indicating that at least some Tγ cells had the OKT3 marker (Moretta et al., 1981). In addition monocyte specific markers other than OKM1 have not been detected on these cells (Haynes et al., 1981). Callard and Beverley (1981) have reported that up to 10% of E-rosette positive cells were not functional, mature T cells. These E^+T^- cells had no other T cell markers, did not respond to mitogens, and were not involved in either helping or suppressing an HIR. This subset of E^+ cells did not express high levels of NK activity and were 50-60% Fcγ receptor positive compared to only 2-4% of the E^+ cells

bearing T cell markers (Beverley and Callard, 1981). Separation of human T cell subsets has been accomplished using the distinguishing monoclonal antibodies and flow cytometry (Hoffman et al., 1980).

CTL activity is characterized by the specific lysis of target cells in the absence of exogenous C' or antibody. In addition to their distinguishing cell surface markers, T cells possess antigen specific recognition structures for tumour antigens (Stulting and Burke, 1973) and alloantigens (Wekerle et al., 1973) which in turn initiate the lytic cycle. Target cell lysis can be divided into three phases; i) recognition, ii) post-recognition, and iii) target cell disintegration (Matter, 1979).

Binding and recognition of the target by the effector initiates cytolysis (Berke and Amos, 1973; Cerottini and Brunner, 1974). Target antigen recognition is specific and reversible, and can take place at both 4°C and 37°C (Wagner and Rollinghoff, 1974) though the phenomenon exhibits some temperature dependence (Berke and Gabison, 1975). Conjugation of the effector and target can be prevented by various metabolic inhibitors (Berke and Gabison, 1975). Cytochalasin B, which affects microfilament formation, has been shown to reversibly prevent intimate contact between the target cell antigens and the effector lymphocyte receptor sites (Plaut et al., 1973). The junctions of immune lymphocytes and target cells show

increased ATPase activity whereas monocytes and non-immune lymphocytes in contact with target cells do not (Penfold and Jones, 1979). Binding ability can also be affected by low pH, EDTA, and Mg^{++} concentration (Stulting and Berke, 1973).

During the post recognition phase primed effector cells can generate target cell damage as early as 30 seconds after exposure (MacDonald, 1975). This stage is where the actual CTL mediated lysis begins and the process is temperature dependent. While binding can occur at both $4^{o}C$ and $37^{o}C$, cytolysis can only occur at the higher temperature (Berke and Amos, 1973; Berke and Gabison, 1975). During this phase DNA synthesis is not necessary but protein synthesis is (Henney, 1973). Colchicine, an agent that prevents microtubular aggregation, irreversibly inhibits cytolysis by preventing the formation of desmosomes at the points of contact between the effector and target cells (Henney, 1973; Plaut et al., 1973). Originally it was thought that the effector cell changed the local charge on the surface of the target cell resulting in a change in the trans-membrane potential and subsequent cell membrane damage (Berke and Amos, 1973). The target then proceeded to an effector independent stage where small molecular weight molecules were released, followed by the larger molecular weight fractions (Ferluga and Allison, 1974). This lead to the conclusion that the target cell lysis was the result of a colloid-osmotic process and leakage of the cell contents

through lesions in the cell membrane (Henney, 1973).

Recent evidence suggests that, although target cell lysis through a colloid-osmotic process does occur, the actual mechanism for the signalling of target cell death is received either at the mitochondrial or nuclear level (Matter, 1979). Upon contact, projections from the T cell enter into the target cell pushing the organelles aside and possibly indenting the nucleus. Although there is an increase in enzymatic activity at the site of contact there is no net flux of ions or tight junction formation (Sanderson and Glauert, 1979). Early nuclear disintegration and mitochondrial disruption occur leading to eventual blebbing of the target cell and subsequent cell death (Sanderson and Glauert, 1979; Russell and Dobos, 1980). Throughout the entire process the effector cells are preserved (Berke et al., 1972) and they have the ability to kill more than one target cell without a reduction in cytolytic capacity (Brunner et al., 1970).

The third stage of cytolysis, target cell disintegration, proceeds independent of additional effector-target interactions (Berke et al., 1972) and is irreversible (Wagner and Rollinghoff, 1974). The killer cell independent lysis, although less temperature dependent than earlier stages of the lytic process, has a longer half-life as the temperature decreases (Martz and Benacerraf, 1975). This may indicate that metabolic events are important during this final phase but it is possible

that the lower temperatures cause the cytoplasm to gel thus slowing down the disruption process (Martz and Benacerraf, 1975). The breakdown of cellular membrane and intracellular components may be due, in part, to release of the target cell's own proteolytic enzymes which could be inhibited at the lower temperatures.

3. Generation of CTL

In order for the CMIR to carry out its surveillance function, by removing foreign antigens through a destructive effector mechanism, it is important that these antigens be recognized as foreign and that the proper effector cells be activated to facilitate removal. The foreign cell surface antigens recognized by the responding effector cells during an anti-alloantigen response were termed histocompatibility antigens (Snell, 1948). They were first described in the murine system by Gorer (1937). The genes coding for the antigens were part of a multi-allelic system (Snell et al., 1953) designated the H-2 system and these genes resided in a region on chromosome 17 known as the Major Histocompatibility Complex (MHC). The first human leukocyte antigens (HLA) were discovered twenty-two years after the H-2 antigens by Jean Dausset (1958) and subsequently mapped to an MHC supergene located on the sixth chromosome (van Someren et al., 1974; Third Conference on Human Gene Mapping, Baltimore, 1975).

The alleles of the H-2 histocompatibility antigens were

originally uncovered through serological methods (Gorer and Mikulska, 1954). These were called the serologically defined (SD) antigens and designated H-2K and H-2D (Klein and Schreffler, 1971). Three similar SD loci, the HLA-A , HLA-B, and HLA-C, have been mapped in the MHC of man (Terasaki, 1970).

The in vitro correlate of the recognition phases of allograft rejection was originally detailed by Bach and Hirschorn (1964). Peripheral blood lymphocytes were taken from two unrelated individuals to test the compatibility of donor-recipient pairs. When the lymphocytes were cultured together they underwent extensive division and morphological transformation. There was also a correlation between the degree of response and the degree of cross-reactivity of allografts from these pairs placed on a previously sensitized unrelated recipient. The recognition phase and its subsequent proliferative response was called the Mixed Lymphocyte Reaction (MLR) or Mixed Lymphocyte Culture (MLC). If cells were cultured in an MLC for several days, CTL developed capable of eliciting a cytotoxic response to ^{51}Cr labelled target cells carrying the histocompatibility antigens to which they were sensitized. This phenomenon occurred in both murine (Hayry and Defendi, 1970; Hodes and Svedmyr, 1970; Cerottini et al., 1974) and human (Solliday and Bach, 1970; Bach, 1973; Sondel et al., 1975) systems. The MLC assay provided a representative in vitro system for the recognition (afferent) stage of the CMIR while the

cell mediated lympholysis (CML) assay which measured the cytolytic capacity of the effector cell to lyse ^{51}Cr labelled target cells (Holm and Perlmann, 1967; Brunner et al., 1968), provided the in vitro analogue to the efferent portion of the CMIR.

The MLC reactivity in murine cultures is abrogated by treatment with anti-theta serum and C' (Plate and MacKenzie, 1973), therefore the cell responding during recognition of the antigens is a T cell. It also bears the Lyl^+ marker but differs from CTL in that it is not $Ly23^+$ (Cantor and Boyse, 1975b). Using B cell depleted lymphocytes in a human system Sondel et al. (1975) showed that the MLC and the CML develop normally. Together these observations suggest that, in the two systems, the afferent and the efferent phases of the CML response are T cell functions.

During studies of the MLR Amos and Bach (1968) noted that SD antigen-identical siblings could in some cases stimulate each other when placed in culture. It was suggested that a recombination event had occurred in the MHC between the HLA-A and HLA-B loci and an unknown MHC antigen coded locus, allowing MLR activity similar to that seen in SD identical but unrelated individuals. The MLC locus was genetically distinct from the SD loci (Bach and Segall, 1972; Eijsvoogel et al., 1972). The MLC activation locus was difficult to detect serologically but did cause proliferative responses and was called the Lymphocyte Defined (LD) locus (Bach et al., 1972a). It was confirmed

that the LD locus was principally responsible for MLR and that the LD alleles, though closely linked to the SD loci, were genetically separable entities and did not always associate with the same SD haplotype (Suciu-Foca and Dausset, 1975).

Using congenic strains of mice carrying intra-H-2 recombinant chromosomes, it was established that the MLC locus was genetically distinct from the SD loci and was located in the I region of the MHC (Bach et al., 1972b). Subsequent investigation showed that the H-2D end differences resulted in a weak stimulation and that an LD-1 locus was associated with the H-2D alleles (Widmer et al., 1973). Another more strongly reactive locus was closer to the H-2K locus but within the I region genes (Abbasi et al., 1973; Meo et al., 1973; Widmer et al., 1973). Aside from the H-2 associated LD locus there is another locus, termed the Mls, which segregates independently of the H-2 and can stimulate a proliferative event (Festenstein, 1974; Schendel and Bach, 1975).

The LD responsive cells provide help during the recognition process and activate SD antigen responsive CTL (Bach, 1973; Wagner, 1973). The T cell response to LD differences also amplifies the CTL anti-SD response, the addition of more LD responsive cells to the culture resulting in a synergistically increased response greater than the sum of the two separate responses (Wagner, 1973). During MLC activation both $Ly1^+$ and $Ly23^+$ cells

proliferate. The Lyl^+ cells recognize immune region associated (Ia) antigens and amplify the response of $Ly23^+$ CTL (Cantor and Boyse, 1975b). Proliferation of a precursor T cell population that differentiate into CTL during in vitro MLR has been demonstrated by Cantor and Jandinski (1974). The characterization of the interaction of Lyl^+ cells with CTL was made possible by the delineation of the Qa1 antigen which could define subsets of the Lyl^+ helper cells (Cantor and Gershon, 1979). Lyl^+ cells, in conjunction with the non-T cells (ie. macrophages), initiate the response. Lyl^+Qa1^- cells stimulate the precursor cells in a $Ly123^+$ pool to differentiate into $Ly23^+$ CTL. Regulation of the response is carried out by a Lyl^+Qa1^+ cells that activate $Ly23^+$ suppressors from the $Ly123^+$ pool, which feedback inhibit the Lyl^+-non-T cell interaction, as well as the effects of the $Qa1^+$ and the $Qa1^-$ cells (Cantor and Gershon, 1979). Promoter factors, which interact with non-T cells, are also released by the helper cells (Golstein et al., 1980). The non-T cell is required to either boost the primed helper cells or cause the action of the promotor factors, that in turn stimulate cytolytic precursor cells, resulting in polyclonal T cell activation and CTL formation. The SD and LD differences have a synergistic effect leading to enhanced cytotoxicity against SD different target cells regardless of the LD phenotype.

Reports that the LD locus was important in graft survival (Sasportes et al., 1972; Cochrum et al., 1973)

prompted investigators to develop assay systems to further characterize the diversity of the LD alleles and to aid in clinical transplantation studies. Use of an unprimed panel of lymphocytes (Netzel et al., 1975) was advantageous in laboratory trials but not in clinical settings since the MLC response took three to four days to develop fully. Lymphocytes from members of families were paired and cultured to recognize specific LD antigens providing primed lymphocytes for a rapid detection assay (Sheehy et al., 1975). Van Rood et al. (1975) recovered antibodies from human serum that were able to inhibit the MLC reaction in a modified MLC inhibition test. It was speculated that the LD determinants were serologically detectable using modified assay systems. Further work has been done in this system, now called the DRw, but it is not known whether the antibody defined specificities are the same as those recognized by the MLC reactive T cells. There is evidence to suggest that the determinants recognized in the H-2 system by proliferative assays and antibodies are distinct entities (Peck et al., 1980).

MLC activation by differences at the LD locus generates effector cells specific for the SD antigens, although under certain conditions a positive MLC may not be prerequisite in humans (Mawas et al., 1973). Cytotoxic cells generated in the absence of a proliferative MLC response can react in CML with the same level of cytotoxicity as CTL that have undergone a strong MLC. A CML stimulation locus distinct

from the HLA-D has been proposed (Long et al., 1976). Similarly just as the LD loci may not be the only one responsible for eliciting a positive MLC, the SD antigens may not be the only targets for the CTL (Eijsvoogel et al., 1973; Bach et al., 1976; Long et al., 1976).

4. Regulation of the CMIR

T lymphocytes display considerable heterogeneity, not only with respect to cell surface markers, but also with respect to effector function (Cantor and Gershon, 1979; Devens et al., 1979). The regulation of a T cell mediated response involves a complex web of interactions between effector and regulatory T cells, non-T cells, and soluble products produced by all of these cells. The first regulatory T-T cell interaction was demonstrated by Cantor and Asofsky (1970) using F1 hybrid recipients. Spleen or lymph node cells added to thymocytes acted synergistically to induce a GvH reaction greater than the sum of the separate activity of the cell populations. It was apparent that two different cell populations participated in the GvH response. Expression of the enhanced response was dependent on a critical ratio of the two cell types.

In vitro studies of the MLC-CML system confirmed that there were T cell subsets that were necessary to enhance the development of CTL. The LD differences were closely linked to the SD differences. LD differences were necessary to generate CTL directed at the SD differences in

human (Eijsvoogel et al., 1972) and murine systems (Alter et al., 1973). Separation of human lymphocyte subpopulations on allogeneic adherent cell monolayers showed that one lymphocyte subtype responded to LD differences by proliferation and that the cytotoxic cells generated developed receptors specific for the SD antigens (Bach et al., 1972a). Wagner (1973) used an in vitro assay system to show that thymocytes responded to LD differences while peripheral T cells reacted against SD. During antigen activation both T cell subtypes pick up the stimulating alloantigen on their surface through specific antigen binding (Nagy et al., 1976a). Treatment with anti-Ly1 serum removed T cells that bound only I region (LD) products, while anti-Ly2 treatment removed the T cells binding K region (SD) products (Nagy et al., 1976b). The reaction to LD had a synergistic effect on the production of CTL. Mouse thymocytes and lymph node cells mixed together gave a response 5-10x higher than the sum of the separate responses during a mixed lymphocyte culture induced cytolysis (Hayry and Andersson, 1974).

Direct evidence distinguishing the different cells involved in the generation of the CTL was reported in the human system using recombinant families (Eijsvoogel et al., 1973), and in the murine system using an elaborate three cell system (Schendel and Bach, 1974). Eijsvoogel et al. showed that sibling responder-stimulator combinations having total HLA identity and MLC reactivity failed to generate a

cytotoxic response against the specific stimulating cell target or against third party cells bearing the same HLA antigens as the stimulating cell. Lack of LD differences failed to yield a response even if SD differences were present. The presence of both SD and LD disparity between responder and stimulator gave a positive cytotoxic response against the stimulating cell target and third party cells sharing common HLA antigens with the stimulator (Eijsvoogel et al., 1973). MLC activation proved to be a prerequisite for differentiation of CTL directed at SD target antigens. Schendel and Bach (1974) selected and tested H-2 congenic mouse strains in various combinations in order to evaluate the ability of different segments of the H-2 to stimulate production of effector cells or serve as targets for lysis. Effector cells mixed with stimulator cells having LD differences only gave an MLC proliferative response but not a CML. Stimulator cells, with SD differences only, did not give an MLC response but did develop a weak CML. A responder cell mixed with a cell bearing LD differences but with SD identity plus a second cell LD identical but SD different in the same culture gave an MLC response and a CML response specific for the SD antigens. The LD and SD determinants did not have to be on the same cell to elicit a response although both were required. The importance of I region disparity in terms of activating the T helper system was demonstrated in vivo and the LD-SD interactions were similar to those found in vitro (Sollinger and Bach,

1976).

Non-T cells can also serve to amplify the action of alloimmune T cells. In the murine system macrophages interact synergistically with T cells to enhance the cytotoxic activity (Jones and Jones, 1978). The interaction is specific and is not genetically restricted. This is in contrast to other findings that both the helper macrophage cells and the killer T cells have clonally distributed receptors for self-MHC molecules, the helper cells having receptors specific for self Ia antigens and exhibiting genetic restriction (Paul et al., 1976).

The synergistic interactions of T cell subpopulations are not restricted to the generation of a CTL. A similar phenomenon has been demonstrated in the proliferative stage of the MLC. Mixtures of lymph node cells and thymocytes from CBA mice result in an increase in the level of proliferation during stimulation by target cells (Cohen and Howe, 1973). Peripheral T cells and lymph node cells are responsible for the proliferative and effector activity while the thymocytes amplify the activation of these cells (Cantor and Asofsky, 1970; Wagner, 1973; Howe and Ghea 1975a; 1975b). Lymphocyte activation and functional enhancement can also be related to the factors or subcellular fractions produced during cellular interactions (Wolstencroft and Dumonde, 1970; Kaldany et al., 1980).

During in vitro cultivation of mouse splenic lymphoid cells another type of regulatory cell develops that

instead of enhancing the CMIR inhibits it. These cultured cells upon sensitization with allogeneic spleen cells were unable to generate CTL and in addition could actively suppress CTL formation by fresh spleen cells (Hodes and Hathcock, 1976). Irradiated, primed lymphocytes could also suppress the production of effector cells in culture (Sinclair et al., 1976a). The suppressive activity was mediated by anti-T cell antibody-sensitive lymphocytes at the sensitization stage of CTL development. The major effect was exerted on the responder cells with a minor, though potentially cytotoxic, effect directed at the stimulator cells (Sinclair et al., 1976b). Recent evidence suggest that there are two subsets of T suppressor lymphocytes. The first, Tsi, suppresses at the induction phase and is capable of binding antigen. The Tsi subset can also induce the formation of Tse cells which can inhibit the response at the effector phase (Clark et al., 1976; Weinberger et al., 1980). As with the soluble enhancing factors mentioned previously there can also be a production of factors that inhibit the CMIR (Ruddle and Waksman, 1976; Granger and Kelb, 1968).

In addition to T cell mediated regulation, products of the HIR can modulate the CMIR. Suppression of the immune response by antibody is achieved either through a simple antigen masking system (Uhr and Moller, 1968) or a more complex system involving regulatory signals from the Fc

portion of the antibody (Sinclair, 1969).

Alloantibodies directed at target cell surface antigens lead to an efferent inhibition of the CMIR by blocking the site of attachment of the CTL (Moller, 1965). Anti-K antibodies will inhibit a T cell response against the K antigens but do not interfere with the response to antigens coded for by the D locus (Lemonnier et al., 1978). This indicates that the antibodies block the T cell response through antigen masking (Sinclair et al., 1975; Lemonnier et al., 1978). Observations made in vivo subsequently confirmed the work done in vitro. Tumour allograft survival was greatly enhanced through the treatment with anti-H-2 antibodies. Anti-K and anti-D sera, as well as anti-I, enhanced allograft survival (Duc et al., 1978). The anti-Ia antiserum was only effective when directed at I region determinants associated with the K locus (Duc et al., 1979). Anti-Ia antibodies inhibit the recognition of the antigenic differences by the amplifier T cells and the anti-K/D antibodies have a peripheral role, either masking or internalizing the corresponding antigens that are recognized by the CTL (Duc et al., 1978). The concentration, the isotype, and the avidity are important in the suppressive effects of the antibody. IgM antibody can enhance tumour allograft survival at low doses but it is inhibitory at high doses. IgG1 is enhancing at any dose (Rubenstein et al., 1974). Enhancement of H-2 incompatible skin grafts was accomplished using anti-I region antibodies, not anti-K or

anti-D (Jansen et al., 1975; Staines et al., 1975). Although the anti-SD antibodies showed only a weak suppressive effect on the CMIR the K end antibodies did exhibit a stronger enhancing effect than the D end antibodies (Staines et al., 1975). All enhancing antibodies isolated contained both IgG1 and IgG2 (Jansen et al., 1975). MLC blocking ability has also been found in the IgG fraction of serum obtained from human allograft recipients (Singal et al., 1975). The blocking antibodies appear to be directed at the DRw antigens, the murine I region equivalent, but their presence does not correlate with the clinical course of the patients.

Fc dependent regulation of an immune response is more complex than antigen masking and involves several cellular components (Sinclair, 1978). Intact antibody is able to inhibit an immune response to a greater degree than $F(ab')_2$ fragments alone (Sinclair, 1969; Sinclair and Chan, 1971) and its suppressive effects do not bear the same dose-response relationship as the fragments (Chan and Sinclair, 1971). Although $F(ab')_2$ fragments can inhibit the generation of an immune response they can not suppress an ongoing response (Chan and Sinclair, 1973). Intact IgG can terminate an established response but its ability to do so can be impaired by the presence of $F(ab')_2$ particles (Chan and Sinclair, 1973). Binding to the antigen is essential for Fc dependent suppression.

Although allograft enhancing antibodies were present in

many recipients it appeared that the T cell susceptibility to antibody suppression was restricted to predominantly antigen masking phenomenon (see above). Studies of Fc dependent antibody suppression indicates that B cells are more sensitive to it than T cells (Hoffman et al., 1974; Oberbarnscheidt and Kolsch, 1978). The lack of T cell sensitivity could be due to inherent genetic resistance, production of factors that abrogate the Fc dependent effect (Sinclair et al., 1976b) or the fact that the T cells recognize antigens in a different conformation than B cells (Benacerraf, 1978).

The IgG fractions of alloantibodies directed against the MHC in rats inhibited the MLC while the $F(ab')_2$ fragments did not (Nielsen et al., 1977). Inhibition occurred regardless of whether the antiserum was directed at the responding or stimulating cells. Similarly Fc dependent regulation of delayed type hypersensitivity and T suppressor cells has been demonstrated (Neveu et al., 1978).

Sinclair and Law (1979) have shown recently that the in vitro CTL response in the murine system can be abrogated through Fc dependent antibody feedback. Allogeneic stimulator cells were modified with trinitrophenol (TNP) thereby providing an antigenic determinant that could be recognized by both B cells and T cells. The addition of anti-TNP antibodies eliminated the response to the alloantigens present on the stimulator cell surface. $F(ab')_2$ fragments did not have the same effect and, since

the alloantigens were not covered up by the anti-TNP antibodies, this suggested that the Fc portion of the antibody was exerting a negative signal that produced a particle specific immunosuppression (Sinclair and Law, 1979).

5. Antigen Receptors on T Cells

It is well established that B lymphocytes use immunoglobulin as their surface receptors but the exact structural nature of the T cell receptor is under considerable debate. Recently investigators have made use of antibodies directed against idiotypic determinants on conventional B cell immunoglobulins and studied the cross-reactivity with molecular structures on T cells that may function as the T cell receptors. Generation of these anti-idiotypic antibodies is based on the fact that an (AxB)F1 hybrid is immunologically tolerant to the alloantigens contributed by both parents. Introduction of either parental cell type (ie. A) into the F1 offspring results in a GvH response (ie. A anti-B). The F1 individual will not respond to the alloantigens on A but the specific A anti-B receptor will be recognized as foreign and a subsequent HIR will be initiated yielding anti-(A anti-B) antibodies (for review see Ramseier and Lindermann, 1972). Using similar reasoning it is possible to generate these antibodies by injecting F1 animals with alloantibodies derived from either parent. F1 antiserum derived using

either of the above methods was capable of blocking the alloreactive T lymphocyte responses against the relevant alloantigen (Ramseier and Lindermann, 1972). These preliminary experiments intimate that the T cell receptor and the B cell receptor (immunoglobulin) have the same idiotypic determinants and therefore must be coded for by the same variable region Ig gene.

The inhibitory effect of the anti-receptor antibodies on T cell alloreactivity was verified by other investigators (Binz et al., 1974; McKearn, 1974). Binz and Wigzell (1975) used reciprocal absorption studies to provide further evidence that the idiotypic determinants of T and B cell receptors specific for the same alloantigen are identical. Absorption of F1 anti-(Lewis anti-DA) antibodies with the IgG fraction of Lewis anti-DA antiserum removed their ability to react with Lewis T cells and absorption with Lewis lymphocytes removed the ability to react with Lewis anti-DA antibodies. Despite this apparent identity, T cell receptors appear to recognize different antigenic determinants on alloantigens (Binz et al., 1979). When purified, soluble MHC polypeptide chains were passed through a column of alloantibodies, the SD determinant and the heavy and light Ia chains from the relevant allogeneic strains were absorbed but none of the self-MHC chains. T cell receptors isolated using the same anti-idiotypic antibodies absorbed the SD determinant, the heavy Ia chain, as well as one heavy self-MHC chain. There was no observable binding

affinity with third party MHC determinants. This indicates that although alloantibodies recognize foreign antigenic determinants there is a requirement for the T cells to recognize the same determinant in conjunction with self-MHC (Paul et al., 1976; Binz et al., 1979). Further characterization of different effector T cell conditions has been done using antibodies specific for the cross-reactive idiotypes (Sy et al., 1980).

T cell receptors share a structurally similar antigen combining site with B cell immunoglobulin. There was speculation that the T cell receptor may be a form of immunoglobulin but the addition of anti-Ig antibodies failed to inhibit either the MLC reaction (Mond et al., 1973) or the CML reaction (Chapuis and Brunner, 1971).

Non-sensitized lymphocytes possess recognition and binding receptors for both allogeneic cells (Feldmann et al., 1972) and syngeneic cells (Wekerle et al., 1973) but lack the killing capacity which must be acquired during sensitization. Incubation of sensitized effector cells on self results in a non-specific absorption of cells whereas incubation on the respective target cells removes only the specific CTL (Golstein et al., 1971). Specific binding by T cells could be inhibited by alloantisera (Hammerling and McDevitt, 1974; Kennedy et al., 1975). The inhibition of binding was restricted only to T cells, B cell antigen binding was not affected, and occurred regardless of whether the alloantisera were directed at the entire H-2 complex or

against K or D end specificities. Recognition of cell surface antigens by unprimed rat T lymphocytes could be blocked by treatment with alloantisera directed against lymphoid cells (Wekerle et al., 1975). Absorption of the blocking alloantisera with non-lymphoid tissue removed those antibodies specific for the SD determinants yet failed to affect the capacity of the antisera to block recognition. The antibodies were recognizing an I region like product that was in close association with the T cell receptor for cell surface antigens (Wekerle et al., 1975).

The T cell receptor shares the same idiotype as the immunoglobulin manufactured in response to the same antigen and this may be coded for by the same V region gene. Despite this, the receptor does not appear to be an immunoglobulin and recognizes antigens in a manner differently than immunoglobulins (Doherty et al., 1977; Benacerraf, 1978; Binz et al., 1979). There is an association between MHC determinants and the T cell recognition sites but this is not indicative of identity, only that a close structural relationship exists and that addition of anti-MHC antibodies results in membrane perturbations that disturb the ability to bind antigens (Benacerraf, 1978).

6. Target Antigens of CMIR

T cell mediated lysis of allogeneic target cells involves the recognition of histocompatibility antigen

differences and the resultant effector function. A CMIR mounted against either virus-infected or chemically modified target cells exhibits an MHC related genetic restriction. During the investigation of the virus-modified system Zinkernagel and Doherty (1974a) infected various strains of mice with lymphocytic-choriomeningitis (LCM) virus and then tested the spleen cells of these strains for their capacity to lyse virus infected C3H strain L cells. The maximum amounts of cytotoxicity exhibited by the effector cells were restricted to target cells either syngeneic or semi-allogeneic to the effector (Zinkernagel and Doherty, 1974a; McMichael et al., 1977). Target lymphocytes from strains differing at the H-2 evoked minimal cytotoxic activity. It had been suggested that the relevant determinants recognized by the CTL are comprised of `altered self' antigens resulting from the interactions between the virus and the MHC determinants (Zinkernagel and Doherty, 1974b). Although the inducing agent for this form of immunity is the virus, the genes involved in this restriction phenomenon are located in the K and/or D regions of the H-2. The target may be modified H-2 antigen, structures coded for by the H-2 complex not normally expressed on the cell surface, or a virus-H-2 antigen complex (Zinkernagel and Doherty, 1975). The self-MHC antigens also play a major role in the structuring of T cell specificities during ontogeny (Fink and Bevan, 1978; Zinkernagel et al., 1978).

Restriction of the cytotoxic response by histocompatibility antigens is not an absolute requirement. In at least one system T cells stimulated by Sendai virus coated syngeneic cells specifically lysed allogeneic target cells (Finberg et al.,1978). Of the sensitized CTL 1-4% of the clones showed this reactivity indicating there must be a high degree of cross-reactivity between allelic variants of the self MHC and the autologous MHC antigens that interact with foreign (ie. viral) antigens.

Chemical modification of the target cells provided the means to study a cell mediated immune response directed against a simple, easily defined molecular moiety. Modification of syngeneic lymphocytes with trinitrobenzene sulphonic acid (TNBS) resulted in a cytotoxic response directed not at the hapten trinitrophenol (TNP) alone, but at the TNP modified self-MHC antigens (Shearer, 1974). This particular system parallels that seen using virus infected target cells. The sensitized CTL only lyse the TNP modified syngeneic cells and never react with TNP modified H-2 non-related congenic target cells (Shearer, 1974). The requirement for H-2 homology was between the modified stimulator cell and the modified target cell. The genetic restriction to TNP resided in the K end in some mice (Shearer, 1974) and in the K and D ends in others (Shearer et al., 1975). The CTL derived specificity was stimulated by both the hapten and associated H-2 antigen since it was possible to block the response with antibodies directed at

either the K and D histocompatibility antigens (Schmitt-Verhulst et al., 1976) or the TNP hapten (Sinclair and Law, 1979).

The intimate association of the TNP hapten and the H-2 antigen has been detailed by Forman et al. (1977a). At a neutral pH the TNBS reacts primarily with free amino groups, (ie. lysine), although there is an interaction with some of the membrane lipids. The addition of the anti-TNP antibodies to TNP modified cells in the absence of C' render the cells resistant to lysis by anti-H-2 antiserum plus C', this presumably is the result of the anti-TNP antibodies blocking the TNP group associated with the antigenic determinant recognized by the CTL (Forman et al., 1977a). There is a direct correlation between the extent of H-2 derivatization and the ability of the cells to act as either stimulators or targets in the CML assay (Forman et al., 1977b). At a concentration of 1 mM TNBS approximately one quarter of the cell surface proteins are TNP derivatized (Forman et al., 1977a). For the activation of the CTL to occur it is necessary for the T cell receptors to recognize a single large site composed of multiple subsites of low and roughly equal affinity. The subsites recognized include, 1) the hapten, 2) the link to the carrier, 3) the amino acid to which it is coupled, and 4) adjacent portions of the antigenic determinants (Janeway, 1976).

In the virus infected cell system there were instances when the modified self sensitized CTL could react with

unrelated allogeneic target cells (Finberg et al., 1978). Early evidence indicated that the TNP system did not exhibit MHC restriction. Sensitization of T lymphocytes to syngeneic TNP spleen cells yielded CTL having cross-reactive cytotoxicity against TNP substituted targets without regard for H-2 compatibility (Burakoff et al., 1976). Antisera directed at K or D antigens on the target inhibited the cytolytic reaction. Use of TNP-chicken RBC's as blockers failed to inhibit. The cytolysis was not TNP specific, only occurring in conjunction with histocompatibility antigens (Burakoff et al., 1976). More recent results suggest that this was not the case and that after stimulation with TNP modified cells the CTL were only capable of lysing those targets having some degree of H-2 homology with the stimulator (Shearer and Schmitt-Verhulst, 1977), although TNP modified target cells expressing any haplotype could be lysed to some extent by effectors generated through allogeneic stimulation (Shearer et al., 1976a).

Twenty to eighty percent of the CTL exhibiting TNP dependent cytotoxicity in the human system showed cross-reactivity that could be directed at common determinants present on all target cells , while the rest were MHC restricted (Shaw and Shearer, 1978). Studies of effector populations from sibling sets showed that forty percent of the activity generated was specific for MHC related polymorphic determinants whereas in unrelated HLA-A and B matched effector-stimulator pairs cytotoxic activity

was directed at TNP in association with relatively non-polymorphic determinants (Shaw and Shearer, 1978). Cross-reactivity was species specific, the effector cells possessing a receptor for TNP in conjunction with cell surface determinants that were widely shared in humans and not in mice (Shaw et al., 1978). As with the murine system the lysis was specific for TNP and a cell surface component since TNP-chicken RBC's or TNP-lysine did not block the reaction.

Lysis of unmodified allogeneic target cells involves recognition of histocompatibility antigens in both the afferent and efferent stages of the CML (Miggiano et al., 1972; Eijsvoogel et al., 1973; Bach et al., 1972a). HLA-D locus incompatibility has been considered a prerequisite (Schendel and Bach, 1974), although not absolute (Long et al., 1976), for the generation of CTL that recognize HLA-A, B, and C locus coded antigens. While it was reported there was no clear division between the antigenic strength of the A and B locus antigens (Kristensen et al., 1975), recent evidence suggests that the B locus antigens are stronger target determinants than the A locus antigens (Eijsvoogel et al., 1976). The HLA-C locus antigens can act as weak target determinants (Grunnet et al., 1976) and although CTL can be generated against DR antigens on Epstein-Barr virus transformed B cell lines (Albrechtsen et al., 1979), such a finding has not been demonstrated using non-transformed lymphocytes as targets (Eijsvoogel et al., 1976; Geha et

al., 1977). There is also evidence that determinants other than HLA-A, B, C, and D/DR antigens can be recognized by CTL.

In the murine system recognition of minor H-2 antigen differences is MHC restricted. The CTL will kill those targets bearing the minor H-2 antigen differences only if the target has the CTL's own major H-2 antigens (Bevan, 1977). This is similar to the self-MHC restricted cytotoxicity observed in the virus modified target cell systems (Finberg et al., 1978). In both these systems, 1-4% of the effectors exhibited some degree of cross-reactivity and were capable of lysing allogeneic targets differing only at the minor H-2 sites, provided the major antigenic structure remained the same. The reactivity may be between allelic variants of self-MHC (Finberg et al., 1978) or may represent a more complex system where the H-2 region codes for a product that interacts with the products of genes that are coded elsewhere to create new, more complex determinants (Bevan, 1975). The H-2 antigen loci may not be the principal target determinants but instead code for modifiers of all other surface components which are the targets in these systems (Bevan, 1975).

Edidin and Henney (1973) showed that the DBA/2 mastocytoma cells stripped of serologically reactive membrane H-2 antigens were susceptible to cytolysis by CTL. A serologically silent product of a non-H-2 linked gene appeared to be the target of the CTL. Cells cultured by

themselves developed spontaneous autologous killing and a cytolytic capacity directed at allogeneic cells (Shustik et al., 1976). This 'promiscuous' cytotoxicity was not caused by a non-specific cytotoxic factor since it could be removed by treatment with anti-theta serum plus C'.

Poor correlations between serological typings and the survival of allografts between unrelated human donor-recipient pairs indicated that the serologically detectable antigens may not be the targets for the CTL (Halgrimson et al., 1971). In some cases cytotoxic cells generated in families exhibited a lesser degree of kill against HLA identical unrelated targets then against HLA genotypically identical targets (Trinchieri et al., 1972) and CTL generated between HLA identical, unrelated individuals exhibited a high level of cytotoxicity against the stimulating cell target (Schapira and Jeannet, 1974). Cytotoxic responses have also been reported in MHC identical sibling transplant pairs (Stiller et al., 1977). In these cases other specificities, distinct from the SD antigens, were the prime targets for the in vitro CML.

Studies performed using allogeneic responder-stimulator combinations against target cells totally MHC distinct from the stimulator cell resulted in a 30% positive cytotoxic response (Mawas et al., 1974). The high frequency of CML directed at non-MHC specificities was seen after both in vitro and in vivo immunizations (Mawas et al., 1974) but SD and LD differences were needed for the enhanced

expression of this phenomenon (Bach et al., 1976). The targets for the CTL have been called the cytolytically defined (CD) antigens (Bach et al., 1977). In some cases the activity directed at the CD antigens can be inhibited by the addition of a more powerful MHC allogeneic stimulator cell (Sondel and Bach, 1976) or killing of third party target cells can arise independent of the blastogenic stimulus used and result in a non-relevant kill, ie. a third party kill occurring in the absence of a specific stimulator kill (Willumsen and Heron, 1974). Involvement of the ABO blood group system, although important as a histocompatibility barrier in vivo, is of little consequence during in vitro sensitization of effectors and subsequent expression of CD antigen directed cytotoxicity (Kristensen et al., 1974a; 1974b). There has been evidence to suggest that the Lewis blood group antigen may be intrinsic to lymphocytes and any incompatibility at this site results in a proliferative response in a primed lymphocyte typing assay (Singal et al., 1981).

CTL directed at apparent HLA incompatibility during an allograft rejection episode were unable to lyse an unrelated individual possessing the same HLA antigens, and were present despite immunosuppression (Grunnet et al., 1975). Cells from patients obtained post rejection were cytotoxic for target cells not sharing the same donor serological mismatches (Gluckman et al., 1977). In vivo produced effector cells were capable of recognizing the CD target

determinants (Mawas et al., 1974; Grunnet et al., 1975; Gluckman et al., 1977).

Recent attempts have been made to type for the CD determinants using specifically sensitized CTL (Kristensen et al., 1976b; 1978). Initial studies involved a minimum number of typing cells selected from histoincompatible donors. The donors were used in the capacity of effector and stimulator cell and were tested against a panel of HLA-A, and B fully incompatible, unrelated target cells. The responding cell populations yielded cytotoxic responses that split the target panel into two clusters (Kristensen et al., 1976a). A larger workshop study performed by Kristensen (1978) used CTL identified as yielding reproducible cytolysis on allogeneic target cells without detectable HLA-A, B, and C antigenic sharing between stimulator and target cells. The effector cells were again tested against lymphoblasts selected at random from the population. Based on a pair-wise comparison between the CTL, three tentative CML defined specificities were recognized. The three groups were defined as having monospecific traits of allelic genetic origin on the basis of a mutually negative correlation and fit to Hardy-Weinberg equilibrium.

MATERIALS AND METHODS

1. Cell Source

The cells used in this study came from human volunteers. The preparation of the cells, condition, cell type and method of procurement varied, depending on the experiment carried out.

1.1) Cells Collected from Normal Donors

Peripheral blood lymphocytes (PBL) were obtained by purification of heparinized whole blood collected in vacuum tubes (Jelco) from normal, healthy volunteer donors. The donors were both male and female and were selected from a panel that had been tissue typed for their MHC antigens.

1.2) Cells Collected from Transplant Recipients and Living Donors

PBL were obtained from recipients both pre-transplant and post-transplant in the course of transplant monitoring. The cells were cryopreserved and stored in liquid nitrogen until the time of the assay. Peripheral blood from living donors was obtained post-transplant, purified and cryopreserved until needed.

1.3) Cells Collected from Family Members of Transplant Recipients

Family members were called in for blood collection and

donated one unit of whole blood into a blood collection bag containing citrate-phosphate-dextrose. The lymphocytes were separated and cryopreserved until needed.

1.4) Cells Collected from Cadaveric Donors

Cells were obtained from spleens of cadaveric donors at nephrectomy. Single cell suspensions were obtained by gentle homogenization of small slices of spleen. The suspension was washed in Hanks' balanced salt solution (HBSS), resuspended in RPMI 1640 and 20% normal human serum, then frozen and stored in liquid nitrogen.

2. Media and Solutions

2.1) HBSS Medium

Hanks' balanced salt solution (HBSS) powder (GIBCO, Grand Island, N.Y.) 9.8 g/l was dissolved in distilled water followed by 0.35 g of $NaHCO_3$. The pH was adjusted to 7.4 prior to filter sterilization.

2.2) PBS Medium

Phosphate buffered saline (PBS) contained 9.6 g/l PBS powder (GIBCO, Grand Island, N.Y.) and 0.01 g/l $CaCl_2$. The pH was adjusted to 7.4 prior to filter sterilization.

2.3) RPMI 1640 Medium

RPMI 1640 powder (GIBCO, Grand Island, N.Y.) 10.4 g/l was mixed with 2 g/l $NaHCO_3$ in distilled water. The medium was supplemented with 100 units/ml penicillin and 100 µg/ml

streptomycin and the pH adjusted to 7.4 prior to filter sterilization.

2.4) Pooled Human Serum

Human A or AB serum was collected aseptically from a panel of normal donors. The individual sera in the panel were tested for anti-human lymphocyte activity and heat inactivated at 56°C for 30 minutes to remove any endogeneous complement activity. The sera were then pooled and stored at -70°C.

2.5) Tissue Culture (TC) Medium

RPMI 1640 medium was supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, and $3x10^{-5}$ M 2-mercaptoethanol. The TC Medium was then supplemented with either 10% or 20% pooled human serum prior to culturing of human lymphocytes.

2.6) Freezing Solution

Freezing solution consisted of 80% (v/v) RPMI 1640, 10% (v/v) pooled human serum, and 10% (v/v) dimethylsulfoxide (Fisher Scientific Co., Fair Lawn, N.J.) added dropwise to the mixture. The freezing solution was kept at 4°C until used.

2.7) Ficoll-Hypaque Solution

Twelve parts of 14% (w/v) of Ficoll (Pharmacia Fine

Chemicals, Uppsala, Sweden) were mixed in distilled water with 5 parts (w/v) of Hypaque sodium diatriozate (Winthrop, Aurora, Ont.). The mixture had a specific gravity (s.g.) of 1.079 and was stored at $4^{o}C$.

2.8) Rosetting Medium

Rosetting Medium (RM) consisted of RPMI 1640 with 2% (v/v) fetal calf serum (FCS) and 2% (v/v) Ficoll.

2.9) Glycylglycine Buffer

Glycylglycine (Fisher Scientific Co., Fair Lawn, N.J.) 22 mg was added to 35 ml of modified barbital buffer.

2.10) Modified Barbital Buffer

Hot distilled water, 250 ml, was added to 2.875 g of barbital then mixed with 1.875 g barbital sodium, 0.083 g $CaCl_2$, 0.238 g $MgCl_2$ and 42.5 g NaCl. The volume was brought to 1 litre and the pH adjusted to 7.4 prior to filter sterilization.

2.11) Cacodylic Buffer

Cacodylic acid (0.276 g in 10 ml of distilled water) was added to 0.438 g sodium cacodylate in 10 ml of distilled water until the pH was 6.9.

2.12) Trypan Blue Dye Solution

Trypan blue dye stock solution contained 1 g trypan blue

(Fisher Scientific Co., Fair Lawn, N.J.) in 500 ml of distilled water. The working solution of trypan blue contained 80% stock trypan blue dye and 20% (4.5%) saline. The stock dye was filtered and kept at 4°C while the working solution was stored at room temperature.

2.13) Trinitrobenzenesulphonate Solution

Trinitrobenzenesulphonic acid (0.054 g) was added to 10 ml of 1.5x PBS. The pH was adjusted to 7.2 with 0.1 N NaOH and the mixture taken to a total volume of 15 ml. TNBS solution was stored at 4°C away from light.

3. Preparation and Purification of Human Lymphocytes

3.1) Purification of Human Lymphocytes

Lymphocytes (either PBL or spleen) were separated on a Ficoll-Hypaque gradient (s.g. 1.079) using the method of Boyum (1969). Eight to ten ml of heparinized whole blood or homogenized spleen was placed in 15 ml Falcon tubes and carefully underlayered with 4-5 ml of Ficoll-Hypaque. After centrifugation at 500 xg for 20 minutes the resulting lymphocyte interface was collected, washed in RPMI 1640 three times, and counted for viability using trypan blue dye exclusion. Before assaying or culturing, the cells were resuspended to the appropriate concentration in tissue culture (TC) medium and either 10% or 20% pooled human A or AB serum.

3.2) TNP Modification of Human Stimulator Cells

TNP modified stimulator cells were prepared by resuspending either purified human lymphocytes or purified murine spleen cells in 1 ml of PBS containing 10 millimoles of TNBS (trinitrobenzene sulphonic acid, Eastman Kodak Co., Rochester, N.Y.) and incubating for 10 minutes at 37°C. After the incubation the cells were washed three times in RPMI 1640 supplemented with either 10% FCS or 10% pooled human serum at 200 xg for 5 minutes. Stimulator preparations were then resuspended to the appropriate concentrations and irradiated (2500 rad) using a ^{60}Co source prior to addition to the cultures.

3.3) Human Target Cell Preparation and Labelling

Four days prior to the assay, frozen or fresh PBL or frozen donor spleen lymphocytes were prepared and resuspended to $1x10^6$ cells/ml in RPMI 1640 and 10% pooled human serum. Targets used in assays involving TNP substitution of either the targets or the stimulators were left to culture for the remaining three days. Phytohaemagglutinin (PHA-Wellcome) was added to all other target preparations to a final concentration of 0.4%, and the cells cultured for three days in a humidified atmosphere containing 5% CO_2 at 37°C. On the day of the assay the target cultures were centrifuged at 200 xg for 10 minutes, resuspended in 1.0 ml of PBS, and 300 mCi of $Na_2{}^{51}CrO_4$ (Amersham-Searle) was added. The mixture was incubated for

1-1.5 hours at 37°C and gently agitated every 30 minutes. The targets were then washed three times in RPMI 1640 at 200 xg for 5 minutes and resuspended to the appropriate concentration in RPMI 1640.

4. Cryopreservation and Recovery Of Human Lymphocytes

Lymphocytes or spleen cells were purified as outlined above then $1-2x10^7$ cells were frozen in glass ampoules containing 1 ml of freezing solution (Section 2.6). Mixing of the cell suspensions and the freezing solution was performed immediately prior to the sealing of the glass ampoules. All the solutions were kept on ice and the freezing solution was added dropwise to the cell suspension. The cells were frozen in a CRYOSON programmed cell freezer, cooling at the rate of 1°C/min from +4°C to -25°C, and then rapidly cooling (5-7°C/min) to -100°C before storage in liquid nitrogen.

Thawing was performed rapidly in a 37°C water bath, and the 10% DMSO cell suspension was quickly transferred to a 15 ml Falcon tube containing 10 ml of cold RPMI 1640 supplemented with 1% human serum. The cells were centrifuged at 200 xg for 5 minutes and washed a minimum of 4 times in cold medium before counting. The total viable cell recovery was determined by trypan blue dye exclusion. Samples with less than 75% viable cell recovery were not used in experiments.

5. The In Vitro Mixed Lymphocyte Culture-Cell Mediated Lympholysis (MLC-CML) System.

5.1) The in vitro generation of CML in the micro system.

Lymphocytes were prepared as outlined above and resuspended in TC medium (Section 2.5) to a final concentration of $3x10^6$ cells/ml. The cells to be used as stimulating cells were resuspended to $1x10^6$ cells/ml then irradiated with 2500 rads γ-irradiation from a ^{60}Co source. The cultures were set up in triplicate in flat bottom Falcon Micro Test II (3040) plates and 100 µl of responder preparation and 100 µl of stimulator were added to each well using Eppendorf pipets. The cultures were incubated for five days at 37°C in a humidified incubator with 5% CO_2 in air. On day 2 of the incubation, 20 µl of pooled human serum was added to each well bringing the total serum concentration to 20%.

On day 5 the targets were labelled as outlined above and resuspended to $1.5x10^5$ cells/ml in RPMI 1640. Twenty µl of target cell suspension was added to each well, using either an Eppendorf Pipet or a Hamilton Multidispenser. Groups were set up to determine the spontaneous and total releases of each target on each tray. For the spontaneous release 20 µl of target suspension was added to 200 µl of TC medium alone without effector cells. The total release was determined by the addition of 20 µl of targets to 200 µl of undiluted Isoterge solution. The trays were incubated for 8 hours at 37°C in 5% CO_2 in air. The plates were then

centrifuged at 500 xg for 10 minutes at 4°C. An Eppendorf pipet was used to transfer 100 µl of supernatant to γ-counting vials. The vials were counted for 4-5 minutes on a Searle Analytical Gamma Counter and the percent specific ^{51}Cr release (PSCR) calculated from the formula:

$$PSCR = \frac{\text{experimental release-spontaneous release}}{\text{total release-spontaneous release}} \times 100$$

5.2) The *in vitro* generation of CML in the Marbrook system

The lymphocytes were prepared as usual and resuspended to 8×10^6 cells/ml. The stimulator cells were inactivated with 2500 rads of γ-irradiation. The cultures were set up in nucleopore sensitization culture chambers developed by Marbrook (1967), and adapted for use in human systems by Fradelizi and Dausset (1975). Briefly the Marbrook chambers consist of an inner glass cylinder, the bottom of which was sealed off by a nucleopore membrane of .45 µ (John's Scientific) maintained by a silicone ring. The effector and stimulator cells were added to this chamber to a total volume of 1 ml. The culture chamber was then inserted into a flat bottom glass outer chamber filled with 10 ml of TC medium. Five hundred µl of effector preparation and 500 µl of γ-irradiated stimulator cells was added to the culture chamber. The chamber was adjusted so the levels of media in the outer and inner chambers were equal. The glass cylinders and reservoirs were corked with foam stoppers to allow gas circulation. The cultures were incubated for six

days at 37°C with 5% CO_2 in air.

On day 6 the effectors were transferred from the Marbrook inner chamber to Falcon 15 ml test tubes (3040) and washed twice in TC medium. They were resuspended in TC to a concentration of $1.5x10^6$ cells/ml and transferred to flat bottom microtitre trays (Falcon) in 200 µl aliquots. All cultures were set up in triplicate. Twenty ul of ^{51}Cr labelled target cells at a concentration of $1.5x10^5$ cells/ml in RPMI 1640. Groups for spontaneous and total releases were included as outlined previously. The cultures were incubated for eight hours then the plates spun at 500 xg for 10 minutes at 4°C and 100 µl of supernatant removed and counted. The PSCR was determined.

6. Separation of Human T Lymphocytes from a Mixed Population

T cells were separated from PBL populations using a modified version of the E-rosette technique as outlined by Jondal et al. (1972). Purified lymphocytes were washed and resuspended to $2x10^6$ cells/ml in rosetting medium (RM). The fetal calf serum used in E-rosetting was previously heat inactivated and absorbed twice with washed SRBC at 4°C and 37°C. Ficoll (Pharmacia, Uppsala) was used to supplement the RM to increase rosette stability. Sheep erythrocytes (Woodlyn Laboratories Ltd., Guelph, Ontario) were stored in Alsever's solution until needed, at which time they were washed three times in RPMI 1640 plus 1% FCS, and resuspended in RM to a final concentration of 0.5% (v/v) using packed

cells.

Four ml of lymphocyte preparation was mixed with 4 ml of SRBC preparation in a 15 ml Falcon test tube and incubated for 15 minutes at 37°C in a humidified 5% CO_2 air incubator. The mixture was then centrifuged at 200 xg for 10 minutes then placed on ice and incubated for one hour at 4°C. The pellet was gently resuspended by rocking on a mechanical platform (Ames Aliquot Mixer) and a minimum of 200 lymphocytes counted to determine the percentage of SERF+ (T) cells. Lymphocytes with three or more erythrocytes adhering were considered to be rosette forming.

To recover the SERF+ and SERF- cells the mixture was underlayered with Ficoll-Hypaque (s.g. 1.079) and centrifuged at 500 xg for 20 minutes. The interface cells (SERF-) were collected and the remaining supernatant discarded. The pellet was then resuspended in 3 ml of sterile distilled water for 20 seconds to remove the SRBC through osmotic shock. Three ml of 0.3 N NaCl was added to restore the osmolarity of the solution. Both SERF- and SERF+ cells were washed twice in RPMI 1640 to remove erythrocyte debris and residual RM.

7. Preparation of Anti-TNP-KLH Antibody

7.1) Preparation of TNP-KLH

Approximately 1 g of ammonium sulphate precipitated keyhole limpet haemocyanin (KLH) (Pacific Bio Marine Laboratories, Venice, California) diluted in 0.9% saline was dialyzed against two liters of saline for three days. It was then centrifuged at 30,000 rpm for 2 hours with a Ti50 rotor in a Beckman L2-65B Ultracentrifuge (Beckman Instruments, Inc., Palo Alto, California). The supernatant was discarded and the sediment dissolved in saline, stirring gently with a glass rod, to a final concentration of 10 mg KLH/ml of saline. Fifty-four mg of trinitrobenzene sulphonic acid (TNBS) were dissolved in 6.75 ml of PBS and the pH adjusted to 7.2. The KLH was slowly added to the TNBS (370 mg KLH + 80 mg TNBS in 10 ml of PBS) then shielded from light and stirred at room temperature for one hour. This mixture was dialyzed against 2 changes of PBS for three days. After dialysis the mixture was centrifuged at 30,000 rpm for 2 hours and the supernatant discarded. The pellet was redissolved in 10 ml of PBS. Insoluble residue was removed by centrifuging at 570 xg for 5 minutes and the supernatant collected. The preparation was dialyzed against PBS for 48 hours. The protein content was determined using the Lowry technique and the TNP content determined by taking the optical density at 360 nm. From these figures the molar ratio of TNP to KLH was calculated and determined to be 60:1 in [redacted] preparations. Methiolate was added as a

preservative and the preparation stored at 4°C.

7.2) Anti-TNP-KLH Antibody Production in Humans

A male volunteer was injected subcutaneously in the arm with 0.1 mg of TNP-KLH, without the use of adjuvants, in a 0.1 ml dose. Seven days after the injection 50 ml of peripheral blood was collected in a non-heparinized blood collection tube. The blood was allowed to clot at room temperature for two hours. The clot was dislodged, the sample centrifuged at 1000 xg for 20 minutes and the clear supernatant collected. The serum was heat inactivated at 56°C for 30 minutes to remove any endogenous complement activity before being stored at -70°C.

7.3) Anti-TNP-KLH Antibody Production in Rhesus Monkeys

Two Rhesus monkeys, weighing approximately 10 kg, were used for the generation of simian anti-TNP-KLH antibody. The monkeys were anesthetized with ketamine hydrochloride (Rogar, Div. of BTI Products Inc., London, Ontario) at a dose rate of 10 mg/kg of body weight injected intramuscularly. They were then injected subcutaneously on their backs with 1 mg of adjuvant free TNP-KLH/monkey in ten 0.1 ml doses. This was repeated on day 7, day 14 and day 21. On days 28, 29, 30, 31, and 32 twenty ml of peripheral blood was collected in non-heparinized blood collection tubes and allowed to sit at room temperature for two hours. The samples were centrifuged at 1000 xg for 20 minutes and

the supernatant removed. The serum was heat inactivated at 56°C for 30 minutes and stored at -70°C.

7.4) Anti-TNP-KLH Antibody Production in the Mouse

CBA mice were injected subcutaneously in the leg with 0.1 mg of TNP-KLH/mouse in complete Freund's adjuvant (CFA) 1:1 by volume in 0.2 ml doses. The injection was repeated 10 days later and the first bleeding performed seven days after the second immunization. Approximately 0.5 ml of blood was collected from each mouse by retro-orbital bleeding with a sharp Pasteur pipette. The mice were boosted every second week with 50 mg of TNP-KLH without CFA and regular bleedings performed between the booster injections. The collected antisera were either left overnight at 4°C or for thirty minutes at room temperature, then the coagulated blood dislodged, and centrifuged at 1000 xg for 30 minutes and the supernatant collected. After centrifugation the sera were heat inactivated by placing in a 56°C water bath for 30 minutes to remove any endogenous complement activity. The antisera was stored at -70°C.

7.5) Titration of Anti-TNP-KLH Antibody

The amount of antibody produced during each immunization procedure was determined by observing the haemagglutination and haemolysis titres of the collected antisera.

7.6) TNP Modification of Sheep Red Blood Cells (SRBC)

Three ml of SRBC in Alsever's solution (Woodlyn Laboratories Ltd., Guelph, Ontario) were washed three times in HBSS (570 xg for 10 minutes) and 0.25 ml of packed erythrocytes resuspended in 2 ml of TNBS in cacodylate buffer (0.83 g TNBS/ml of buffer). The cells were shielded with foil and gently swirled for 10 minutes. They were centrifuged and resuspended in 10 ml of glycylglycine buffer and left to stand for 10 minutes at room temperature. The TNP-SRBC were then washed once with glycylglycine buffer and three times with HBSS and the resultant packed pellet was diluted to 0.5% packed volume in the suspending medium for use in the haemagglutination and haemolysis assays.

7.7) Haemagglutination Assay

Step-wise serial dilutions (25 µl aliquots) of the antisera to be tested were made in V-bottom microtitre plates (Cooke Engineering Co., Alexandria, Virginia) using PBS with 1/200 normal rabbit serum. Twenty-five µl of TNP-SRBC control SRBC in buffer were added to the wells of the respective dilution series. Two controls, TNP-SRBC in buffer and SRBC in buffer, were included. The mixture was incubated at room temperature for two or more hours before the results were visually evaluated. Individual wells were scored as +, +/-, or - to indicate haemagglutination, partial haemagglutination or no haemagglutination.

7.8) Haemolysis Assay

A duplicate dilution series of the antisera to be tested was done with HBSS (100 µl aliquots) in 6x50 mm disposable glass tubes (Kimble, Div. of Owens-Illinois, Vineland, N.J.). Ten µl of TNP-SRBC or control SRBC were added to each tube and gently mixed. Duplicate controls of TNP-SRBC and SRBC in buffer alone were also included. The tubes were then incubated at 37°C in a humidified atmosphere with 5% CO_2 in air for 30 minutes. At the end of the incubation period, 90 µl of "Hemo-Lo" guinea pig complement (Cedarlane Laboratories Ltd., Hornsby, Ontario) was added to each tube, including one of the SRBC and TNP-SRBC controls. HBSS was added to the other two controls. The cultures were incubated for 45 minutes before the results were read. The amount of haemolysis in any tube was evaluated by visually comparing the amount of clearing in the tubes against the controls.

8. Statistical Methods

Details of standard statistical methods used in this thesis, such as standard deviation, standard error, and contingency tables, can be found in `Biostatistical Analysis' by Zar (1974).

The form of analysis termed Interaction Analysis was originally developed by Takasugi and Mickey (1976). Briefly this form of analysis allows quantitative comparison of results across many target cells with different

sensitivities. It evaluates the effect of one lymphocyte suspension on several targets and also the effect of different lymphocyte samples on one target. Use of this method allows the separation of selective cytotoxicity from non-selective cytotoxicity within a matrix. The selective and the non-selective cytotoxicities are calculated from the results of an array of tests and are placed in a two by two table in which the rows correspond to the effector cells and the columns correspond to the target cells. The following equations were used to calculate the two forms of cytotoxicity;

$$\text{Selective Cytotoxicity} = T - E_A - T_A + O_A$$

$$\text{Nonselective Cytotoxicity} = E_A + T_A + O_A$$

where:

T=percent cytotoxicity for each effector-target combination

E_A=average test result for each effector against different targets

T_A=average test results for a target cell with different effectors

O_A=overall average for all the tests

RESULTS

1. Characteristics of Cytotoxic Lymphocytes Directed against Haptenic Antigens on the Surface of Autologous and Allogeneic Cells

1.1) In vitro generation of a human cytotoxic cell to trinitrophenyl modified autologous cells.

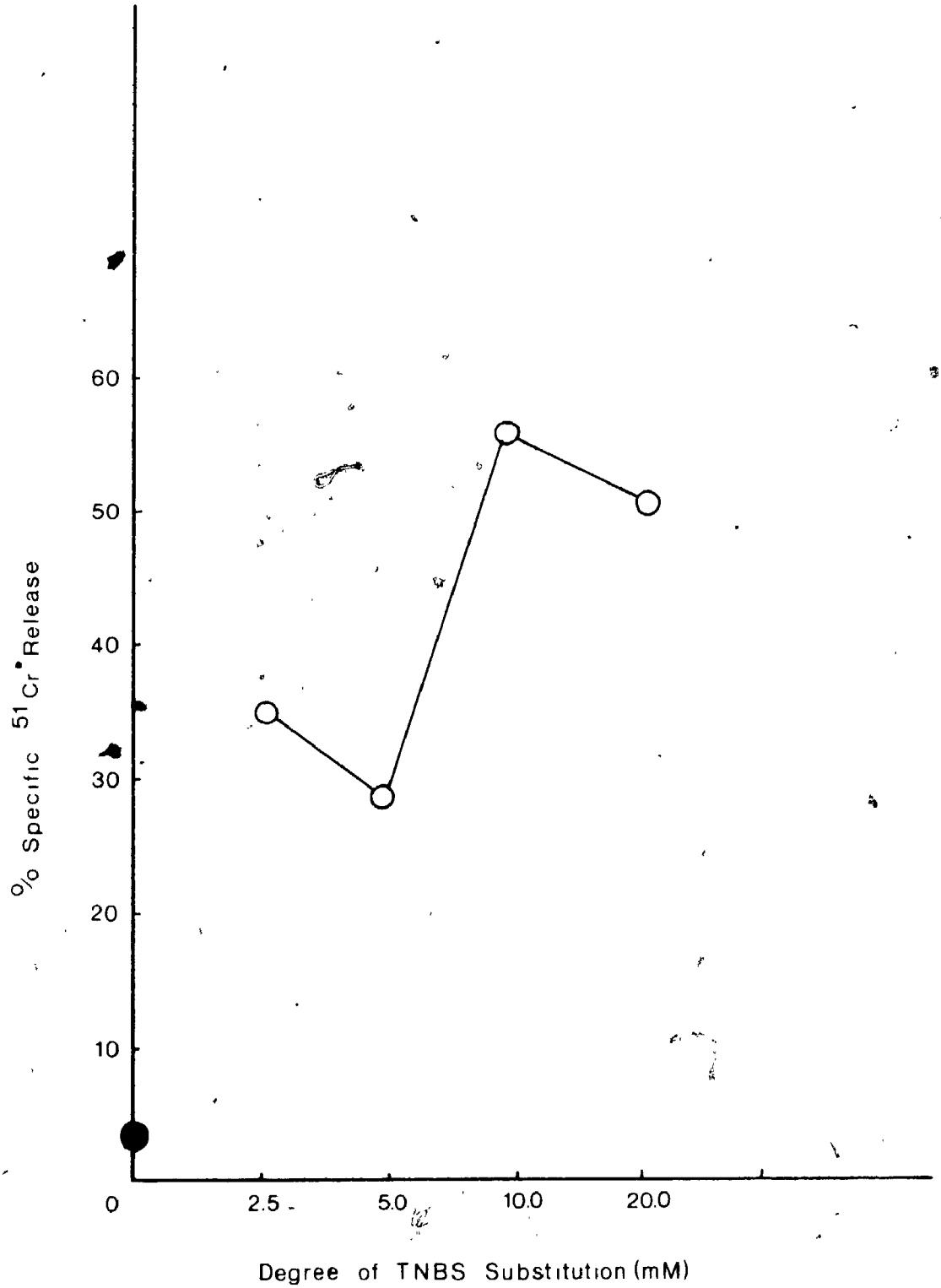
The in vitro generation of TNP altered-self reactive human CTL in both the micro and macro tissue culture systems requires some form of `help' for the differentiation to take place. In the micro system the presence of allogeneic cells or antigen activated culture supernatants were required for the response (Friedman et al., 1978; 1979), while a secondary stimulation was necessary for CTL activation in the macro system (Shaw et al., 1978).

PBL's were obtained from normal human donors and used in Marbrook tissue culture systems in the capacity of either responder cell, stimulator cell, or target cell. The stimulator cells were substituted with TNP using varying concentrations of TNBS. These cultures were incubated at 37^{o}C and on day 6 the sensitized CTL were washed, aliquoted into microtitre trays and ^{51}Cr labelled target cells added. The target cells had been modified using 10mM TNBS. Substitution of the stimulator cells with any amount of TNBS stimulated an anti-TNP-modified self response (Fig. 1). A maximum cytotoxic capacity was generated with stimulators modified using 10 mM TNBS. A higher degree of substitution failed to increase the effector cell

Fig. 1

TNP substitution of the stimulators used in the generation of an anti-TNP modified self response

The cytotoxic response of a responder sensitized in Marbrook tissue culture vessels to TNP modified self substituted with TNBS at concentrations ranging from 2.5 mM to 20 mM and then tested against unmodified (●) or TNP modified (O) self targets.


% Specific $^{51}Cr^{\bullet}$ Release
60
50
40
30
20
10
0
2.5
5.0
10.0
20.0
Degree of TNBS Substitution (mM)

capability.

Responder and stimulator cells were varied in terms of absolute numbers and ratios (Table 1). The final volume for culturing in the Marbrooks was always 1 ml. Responder cells were mixed with stimulator cells substituted with 10 mM TNBS and assayed for activity on day 6. Stimulation of the responder with unmodified self did not give a response against the unmodified self target but did yield a small, but significant, response to a TNP modified self target. Sensitization to hapten modified autologous cells resulted in the generation of CTL specific for hapten modified targets (Table 1). Higher concentrations of responder and stimulator did not inhibit the ability to generate a response and in one case (8:2) generated a near maximal response. This activity could be the result of a greater amount of responder-stimulator contact whereas at the higher total concentrations overcrowding occurs so that optimum sensitization does not occur. The highest cytotoxicity was generated in the cultures containing $4x10^6$ responders and $4x10^6$ stimulators per ml and these cell concentrations were used in all subsequent experiments.

1.2) In vitro generation of CTL directed against TNP-modified allogeneic cells

The use of trinitrophenyl-derivatized autologous cells as sensitizing agents allowed the generation of CTL specific for 'modified self', although the antigenic determinant, the hapten group, presents only a portion of the subsite that can

Table 1
Varying Responder-Stimulator Ratios in the Marbrook System and their Effect on the Generation of an Autologous Anti-TNP Response.

EFFECTOR	R:Sx[1] RATIO x 10^6	TARGETS	
		A	A-TNP
A.Ax	4:4	0.0	4.4 ±0.4[2]
A.A-TNPx	8:4	0.0	17.0 ±1.5
	8:2	2.4 ±0.2	23.3 ±1.2
	8:1	3.2 ±0.9	16.9 ±1.5
	4:4	0.0	24.5 ±2.1
	4:2	0.0	17.7 ±2.1
	4:1	0.0	19.6 ±2.3

[1] Responder to stimulator ratio
[2] % specific ^{51}Cr release ± standard deviation

be specifically recognized as foreign by a T-cell receptor (Janeway, 1976).

CTL induced by TNP altered self antigenic determinants were tested against the same hapten on an allogeneic target cell with major histocompatibility antigen differences. There was little non-specific activity against the unmodified allogeneic target cells (Table 2). Significant reactivity was directed at the hapten-modified targets at the R:Sx ratios of 8:2 and 4:4. This corresponded to the ratios exhibiting maximal amounts of cytotoxicity in the autologous system. The ratio of 4:4 was optimum and was used in all the subsequent experiments. In contrast to the reactivity observed at all concentrations and ratios in the TNP-modified autologous system, cytotoxicity was restricted to only the optimal ratios (Table 2).

Purified PBL were sensitized to either autologous or allogeneic stimulator cells substituted with varying amounts of TNBS then assayed in microtitre trays on day 6 against TNP-modified allogeneic target cells. The responder, stimulated by unmodified self, failed to elicit a response. Sensitization to trinitrophenylated self at all levels of derivatization yielded a response directed at the TNP-allogeneic cells. Cytotoxicity rose from a low at a concentration of 2.5 mM TNBS to a peak at 10 mM TNBS (Fig. 2). The cytotoxic response dropped slightly at the highest levels of derivatization. CTL sensitized to the specific unmodified allogeneic stimulating cell gave a low, but

Table 2

Varying Responder-Stimulator Ratios in the Marbrook System and their Effect on the Generation of an Allogeneic Anti-TNP Response.

EFFECTOR	R:Sx[1] RATIO x 10^6	TARGETS	
		B	B-TNP
A.Ax	4:4	0.0	0.0[2]
A.A-TNPx	8:4	0.0	0.0
	8:2	0.0	11.1 ± 2.6
	8:1	0.0	0.0
	4:4	0.0	18.5 ± 11.7
	4:2	0.0	0.0
	4:1	3.2 ± 1.5	0.0

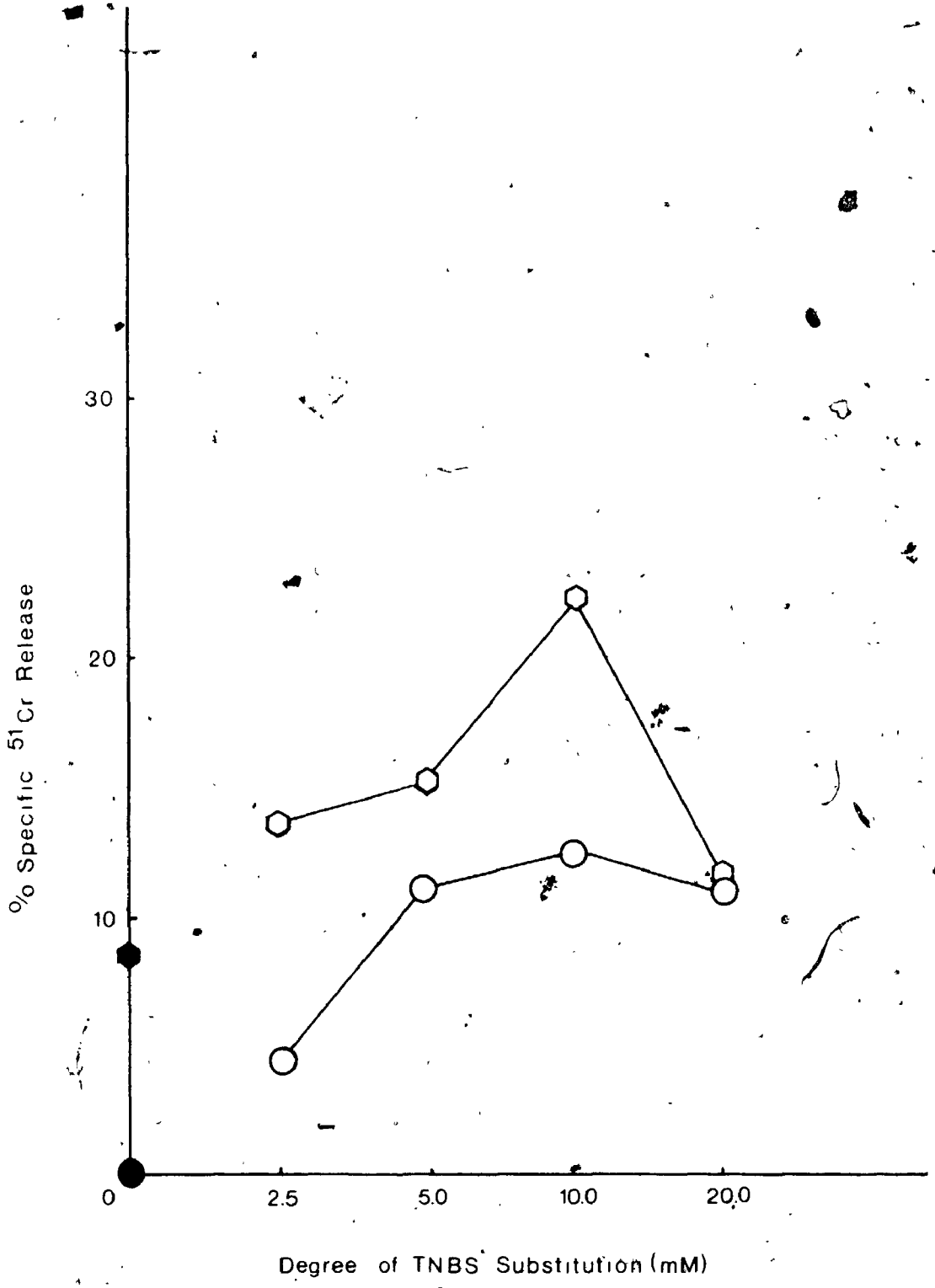
[1] Responder to stimulator ratio

[2] % specific ^{51}Cr release ± standard deviation

Fig. 2

TNP substitution of the stimulators used in the generation of an anti-TNP response against a TNP modified allogeneic target

The cytotoxic response of a responder sensitized in Marbrook tissue culture against unmodified self (●), TNP modified self (○), an allogeneic stimulator (⬢) and a TNP modified allogeneic stimulator (⬡) and tested against the TNP modified allogeneic target cell was determined. The concentration of the TNBS used in the derivatization of the stimulator cells ranged from 2.5 mM to 20 mM.


% Specific ^{51}Cr Release
30
20
10
0
2.5
5.0
10.0
20.0
Degree of TNBS Substitution (mM)

significant, ^{51}Cr-release (Fig. 2). After sensitization to hapten modified allogeneic stimulators there was a higher ^{51}Cr-release than with the unsubstituted stimulating cell-control. The cytotoxic response peaked when the CTL were cultured with the stimulators derivatized with [illegible] mM TNBS. Throughout all other experiments the stimulating cells, whether autologous or allogeneic, were substituted with the TNP hapten using 10 mM TNBS as outlined in the Materials and Methods.

1.3) Effect of allogeneic culture supernatants on the generation of an anti-TNP response

The degree of immunological responsiveness to an antigen can vary from responder cell to responder cell. This is true, not only for allogeneic responses to histocompatibility antigens, but also for a cytotoxic cell response to hapten altered self antigens (unpublished observation). In an effort to induce a cytotoxic response from unresponsive CTL or to enhance the response from minimally reactive cell preparations, the effect of the addition of allogeneic culture supernatants was studied.

Cell free supernatants were obtained from 48 hour cultures containing $5x10^6$ responder lymphocytes plus an equal number of γ-irradiated autologous (control) or allogeneic stimulators. Each supernatant was added, in a final mixture of one part supernatant to three parts normal human serum, to fresh Marbrook cultures of autologous responder cells plus either TNP modified or unaltered

autologous stimulators. Addition of the control supernatant (A.Ax) to a non-responder CTL failed to induce a response regardless of the type of stimulating cell used (Fig. 3). Allogeneic culture supernatants (A.Bx) added to this system facilitated the generation of CTL in response to TNP modified autologous stimulation. The supernatant did not trigger CTL that were capable of lysing B targets. This indicated that the supernatants did not contain soluble alloantigens shed from the stimulator (Bx) during supernatant generation. Of interest is the fact that the same antigen activated supernatants could induce an autologously stimulated CTL to develop a TNP directed response. The response was not significantly higher than the response obtained using allogeneic culture supernatant and TNP modified autologous stimulators.

Control supernatants were added to a poor responder cell preparation. Sensitization to TNP was specific for modified self, although present at a low level (Fig. 4). Addition of alloantigen induced supernatants to TNP modified-self stimulated CTL did not increase the TNP directed response above that exhibited by cultures treated with control supernatants. The allogeneic supernatant (A.Bx) did activate the autologously stimulated responder to elicit a marked TNP cytotoxic response. In both the non-responder (Fig. 3) and minimal responder (Fig. 4) systems the addition of allogeneic culture supernatants induced the activation of

Fig. 3

The effect of allogeneic culture supernatants on the generation of a CTL specific for TNP modified autologous targets

A non-responsive responder cell (A) was co-cultured with autologous (A) or TNP modified autologous (A-TNP) stimulator cells. Cultures were supplemented with control (A.Ax) or allogeneic (A.Bx) culture supernatants (final dilution 1:4). After six days the responders were harvested and tested against unmodified self, TNP modified self, and the specific allogeneic cell used in the culture supernatant generation.

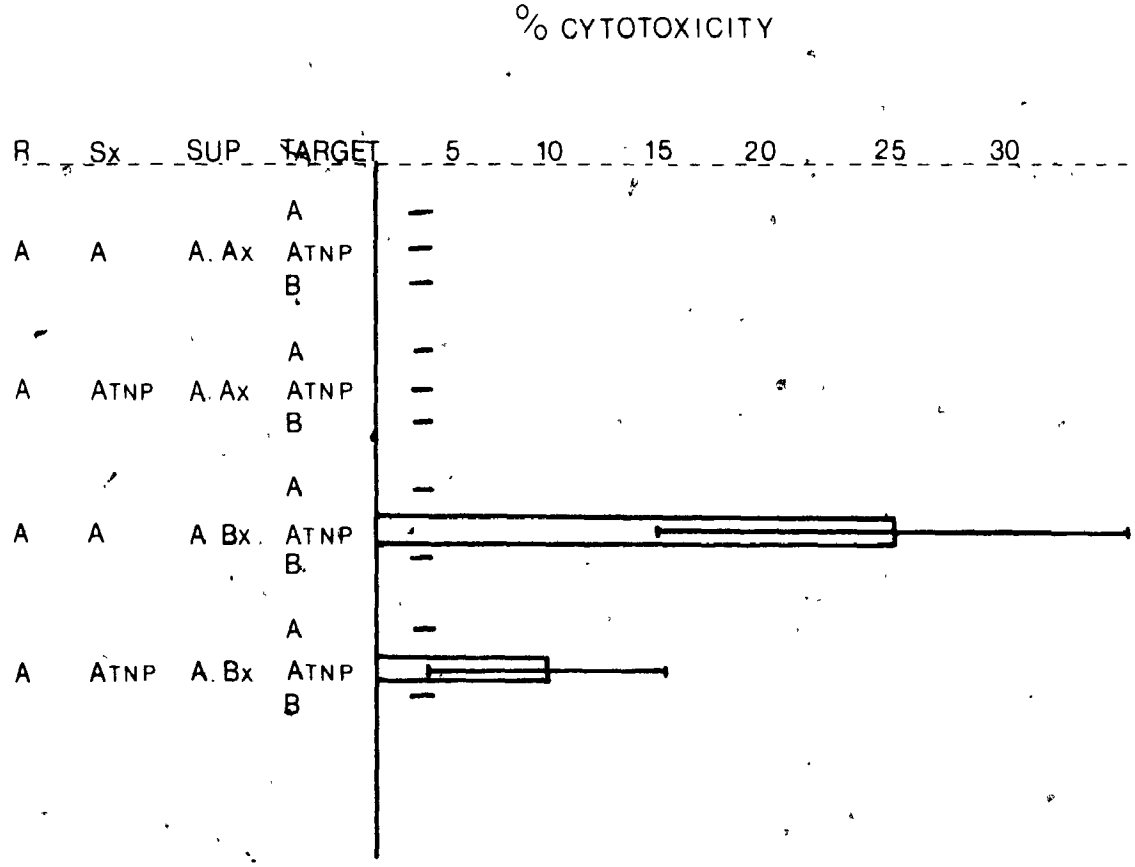
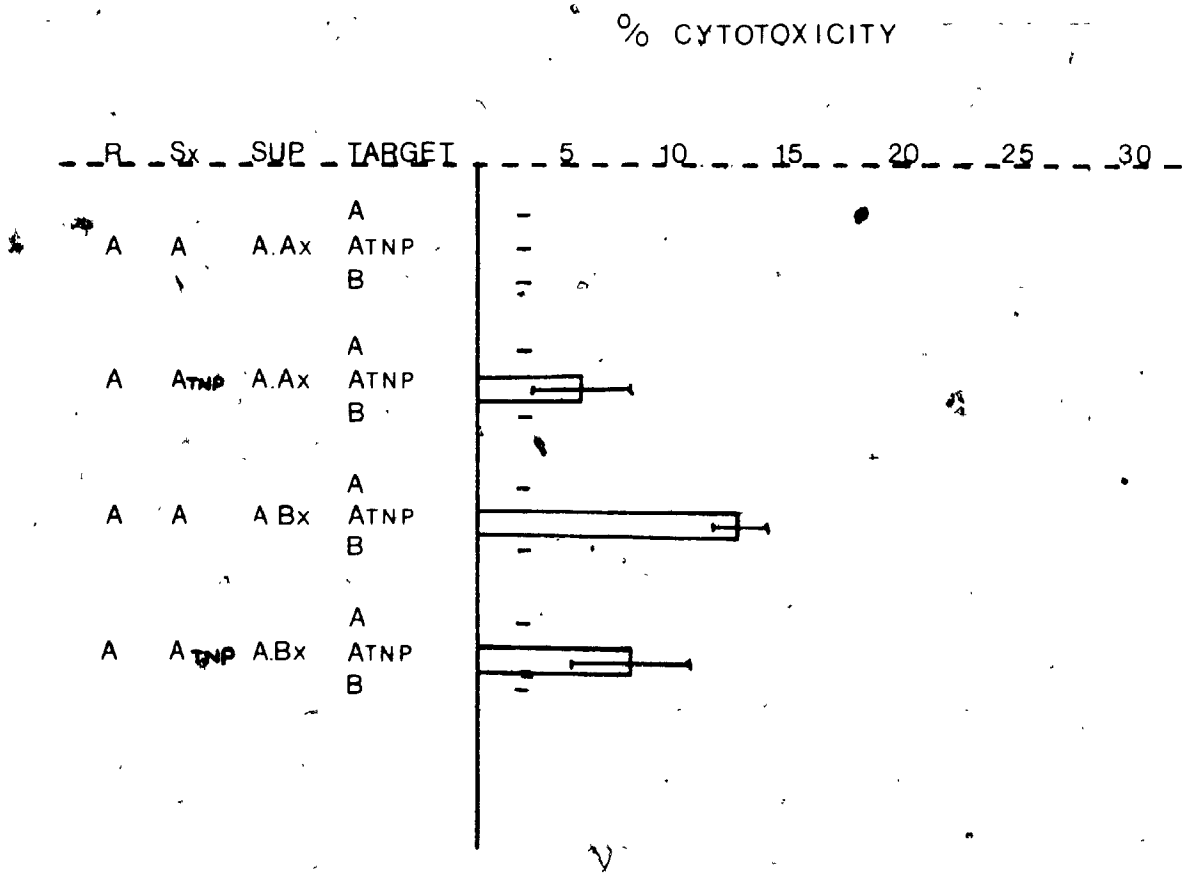

% CYTOTOXICITY
R
Sx
SUP
TARGET
5
10
15
20
25
30
A
A
A. Ax
ATNP
B
A
ATNP
A. Ax
ATNP
B
A
A
A Bx.
ATNP
B.
A
ATNP
A. Bx
ATNP
B

Fig. 4

The effect of allogeneic culture supernatants on the generation of a CTL specific for TNP modified autologous targets

A poor responder cell (A) was co-cultured with autologous (A) or TNP modified autologous (A-TNP) stimulator cells. Cultures were supplemented with control (A.Ax) or allogeneic (A.Bx) culture supernatants (final dilution 1:4). After six days the responders were harvested and tested against unmodified self, TNP modified self, and the specific allogeneic cell used in the culture supernatant generation.


% CYTOTOXICITY
R
Sx
SUP
TARGET
5
10
15
20
25
30
A
A
A.Ax
A
ATNP
B
A
A
ATNP
A.Ax
A
ATNP
B
A
A
A Bx
A
ATNP
B
A
A
A.Bx
A
ATNP
B

CTL capable of reacting with an antigen similar in structure, or identical to, TNP.

1.4) Characterization of the cell involved in anti-TNP responses in the autologous and allogeneic systems

Human PBL's from normal donors were sensitized in Marbrook tissue culture vessels against TNP modified self for six days. The cells obtained were separated on the basis of the ability of human lymphocytes to form spontaneous rosettes with sheep erythrocytes. Unfractionated effectors, SERF+ (T) cells and SERF- (non-T) cells were assayed in the micro system against ^{51}Cr labelled autologous, TNP modified autologous, and TNP modified allogeneic target cells. In sensitized effector cultures the SERF+ fraction made up 65% of the total population. This represents an increase over the 40% SERF+ cells found in normal, unstimulated lymphocyte populations.

Both the T and non-T cell activity were TNP directed since there was no cytotoxic activity directed at the unmodified self target (Table 3). The unseparated, hapten sensitized CTL reacted with the TNP modified self and a TNP modified allogeneic cell. The autologous and allogeneic cells matched across the MHC except at one B locus. Cytotoxicity directed at B-TNP was three times greater than that directed at the modified self. Separation of the effector into the T and non-T cell fractions resulted in a decreased response in the SERF+ fraction and an increased response in the SERF- fraction, compared to the unseparated

Table 3
Separation of Effectors Specific for Hapten Modified Self into Sheep Erythrocyte Rosette Forming Cell Fractions.

EFFECTOR	FRACTION	TARGET		
		A	A-TNP	B-TNP
A.A-TNPx	UNSEPARATED	0.0	10.1[1]	31.7
	SERF+	0.0	3.3	31.3
	SERF-	0.0	14.0	47.9

[1] Percent specific ^{51}Cr release.

Histocompatibility Typing

CELL A 2,24/8,44/-,-

CELL B 2,24/40,44/-,-

control, when assayed on modified autologous cells. There was no change in the activity of the T cell fraction towards the modified allogeneic target but a marked increase in the cytotoxic activity in the SERF- fraction after the separation.

An effector was sensitized to hapten modified self and tested against a hapten substituted allogeneic target cell carrier with a minimum of MHC sharing with the CTL (Table 4). The unseparated effector did not react with the unaltered self but did react with the TNP modified autologous and allogeneic cells. The cytotoxicity against the hapten modified allogeneic cell was less than the cytotoxicity against the TNP modified self target. The SERF+ cells were cytotoxic to TNP modified self but showed no activity against the modified allogeneic target. All the anti-B-TNP activity resided in the non-T cell fraction of the preparation. Reactivity in the SERF- populations was three times greater than in unfractionated populations. Cytotoxicity directed at hapten modified self was evenly distributed between the SERF+ and SERF- fractions. A small degree of self reactivity was directed at the unmodified autologous target by the non-T cell fraction.

In some cases, where reactivity was expected, TNP directed cytotoxicity was observed on the specific modified autologous target but not on the modified allogeneic cell using the unseparated CTL (Table 5). Separation of the effector and subsequent assay against A-TNP gave a cytotoxic

Table 4
Separation of Effectors Specific for Hapten Modified Self into Sheep Erythrocyte Rosette Forming Cell Fractions.

EFFECTOR	FRACTION	TARGET		
		A	A-TNP	B-TNP
A.A-TNPx	UNSEPARATED	0.0	22.1[1]	13.2
	SERF+	0.0	17.7	0.0
	SERF-	7.8	20.7	35.0

[1] Percent specific ^{51}Cr release.

Histocompatibility Typing

CELL A 1,3/14,52/-,-

CELL B 1,24/7,12/4,-/2,-

Table 5
Separation of Effectors Specific for Hapten Modified Self into Sheep Erythrocyte Rosette Forming Cell Fractions.

EFFECTOR	FRACTION	TARGET		
		A	A-TNP	B-TNP
A.A-TNPx	UNSEPARATED	0.0	11.9[1]	0.0
	SERF+	0.0	13.2	6.8
	SERF-	0.0	24.9	17.5

[1] Percent specific, ^{51}Cr release.

Histocompatibility Typing

CELL A 2,28/7,27/2,-

CELL B 24,29/7,27/-,-

T cell fraction as well as a very reactive non-T cell fraction. Manipulation of the CTL population also resulted in the expression of anti-B-TNP responsiveness in both fractions. The SERF- cells exhibited a higher degree of reactivity than the SERF+ cells (Table 5). There was no non-specific activity directed at the unmodified autologous target.

This apparent activation of responsiveness was also observed in reactions to hapten modified autologous targets (Table 6). There was no non-specific responsiveness or TNP specific activity in the unseparated CTL population to either TNP modified autologous or TNP modified allogeneic target cells. Reactivity was restored towards both targets upon separation of the CTL into SERF+ and SERF- populations. Microscopic examination of different fractions of cells involved in the above experiments showed the SERF+ population to be small to medium sized lymphocytes while the SERF- fraction contained a high percentage of granulocytes and large lymphocytes. The high degree of activity associated with this fraction could be the result of derepression of the population by removal of regulatory T cells.

The expression of a response in an unresponsive CTL upon fractionation into the SERF+ and SERF- populations may be the result of removal or segregation of a regulatory cell influence or the result of stimulation through manipulation. Cells were tested upon manipulation without cell subtype

Table 6
Separation of Effectors Specific for Hapten Modified Self into Sheep Erythrocyte Rosette Forming Cell Fractions.

EFFECTOR	FRACTION	TARGET		
		A	A-TNP	B-TNP
A.A-TNP	UNSEPARATED	0.0	0.0	0.0
	SERF+	0.0	25.7[1]	15.1
	SERF-	0.0	62.9	20.1

[1] Percent specific ^{51}Cr release.

Histocompatibility Typing

CELL A 1,-/8,14/-,-

CELL B 2,25/18,35

separation using a Ficoll-Hypaque gradient (Table 7). Effector cells were sensitized in the Marbrook system to TNP modified autologous stimulators and on day 6 washed and resuspended to $3x10^6$ cells/ml. Portions of the cells were overlayed on a Ficoll-Hypaque gradient (D=1.079) and centrifuged at 1700 rpm for twenty minutes. The cells at the interface were then collected, washed and resuspended to the appropriate concentration then assayed in the micro system against ^{51}Cr labelled target cells. Unmanipulated CTL were also assayed. The pellet was discarded.

The TNP sensitized effectors did not react against unmodified autologous or allogeneic targets (Table 7). Effector A did not elicit a response against the specific stimulating cell target (A-TNP) but did give a cross-reactive response to the TNP substituted allogeneic cell. Upon Ficoll-Hypaque fractionation of the viable cells from the dead cells reactivity against modified self was restored. The Ficoll-Hypaque separated CTL reacted to the TNP modified autologous target cell while there was no significant change in the response to the TNP modified allogeneic target. Effector B produced a cytotoxic response to both the TNP modified autologous and the TNP modified allogeneic target cells. Ficoll-Hypaque separation of the cells resulted in a marginal increase in reactivity towards both targets. Separation of the effector cell subtypes was not necessary to restore responsiveness to a TNP directed CTL. During sensitization the γ-irradiated

2

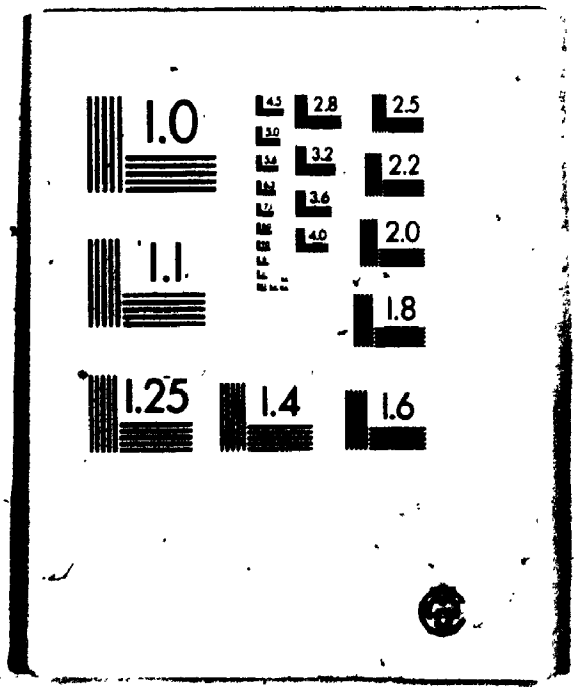


Table 7

Ficoll-Hypaque Separation of Effectors Sensitized to TNP Modified Self

EFFECTORS		TARGETS			
		A	A-TNP	B	B-TNP
A.A-TNPx	UNSEPARATED	0.0	0.0	0.0	22.6[1]
	FICOLL-HYPAQUE	0.0	18.9	0.0	23.2
B.B-TNPx	UNSEPARATED	0.0	26.2	0.0	17.4
	FICOLL-HYPAQUE	0.0	31.1	0.0	21.0

[1] Percent specific ^{51}Cr release.

Histocompatibility Typing

CELL A 2,-/40,51,/2,-/3,-
CELL B 3,-/8,21

stimulating cell population decreases by 90% (Ward and Jevnikar, unpublished observation). Ten percent of the cells still remain. Removal of cell debris and dead cells through Ficoll-Hypaque fractionation resulted in the increase in cytotoxic response in responsive CTL and the restoration of the response in non-responsive CTL.

1.5) Effects of mouse anti-TNP antibody on the immunoregulation of the TNP directed autologous and allogeneic response with human cells

Anti-TNP antibody mediated suppression of a T cell mediated anti-allogeneic in vitro cytotoxic cell response to TNP coupled allogeneic stimulating cells has been demonstrated in the murine system by Sinclair and Law (1979). The effects of passively administered anti-TNP antibodies in the immunoregulation of an in vitro human T cell response were examined. Human PBL's were sensitized to autologous, allogeneic, TNP modified autologous, and TNP modified allogeneic cells. The γ-irradiated stimulators were placed in Marbrook vessels then pooled human serum, normal mouse serum, mouse serum with a high concentration of anti-TNP antibodies, or mouse serum with a low concentration of anti-TNP antibodies was added. The responder cells were subsequently added so that the total culture volume was 1 ml with a 20% final serum concentration. The cells were incubated at 37°C for six days then harvested and washed twice. The CTL were resuspended to 3×10^6 cells/ml and serially diluted into microtitre trays in 10% pooled human

serum plus RPMI. ^{51}Cr labelled autologous, allogeneic, TNP modified autologous, or TNP modified allogeneic target cells were added and, after an 8 hour incubation, the percent specific ^{51}Cr release was determined.

Responders sensitized to allogeneic stimulating cells, in the presence of mouse serum of any type, did not generate a response to the autologous control target (Fig. 5). Similarly the addition of mouse serum, or anti-TNP antibodies, had no effect on the ability to mount a CTL response to MHC antigens (Fig. 5) when tested against the specific stimulating cell target.

Stimulation of a responder with TNP modified allogeneic cells resulted in some autoreactivity against the autologous target in the absence of haptenic modification (Fig. 6). This low level non-specific activity was present in all fractions regardless of the type of serum added to the Marbrook. The addition of mouse serum, or anti-TNP antibodies, had no effect on the responder's ability to mount an anti-allogeneic response (ie. anti-B). The specific anti-B-TNP response in human serum was higher than the anti-allogeneic response, thus effectors recognize modified as well as unmodified alloantigens. The addition of anti-TNP antibodies at any concentration caused a slight decrease in the response but not any more than the normal mouse serum control (Fig.6). The antibodies did not specifically inhibit the response.

In Figure 7, the effects of anti-TNP antibody on CTL

Fig. 5

The effect of murine anti-TNP antibody on an anti-allogeneic response

A human responder cell was sensitized to an allogeneic cell in the presence of normal human serum (hexagons), normal mouse serum (circles), murine anti-TNP antibody (triangles), and murine anti-TNP antibody diluted 1:100 with normal mouse serum (squares) and assayed against an autologous target (closed symbols) and the specific stimulating allogeneic target (open symbols).

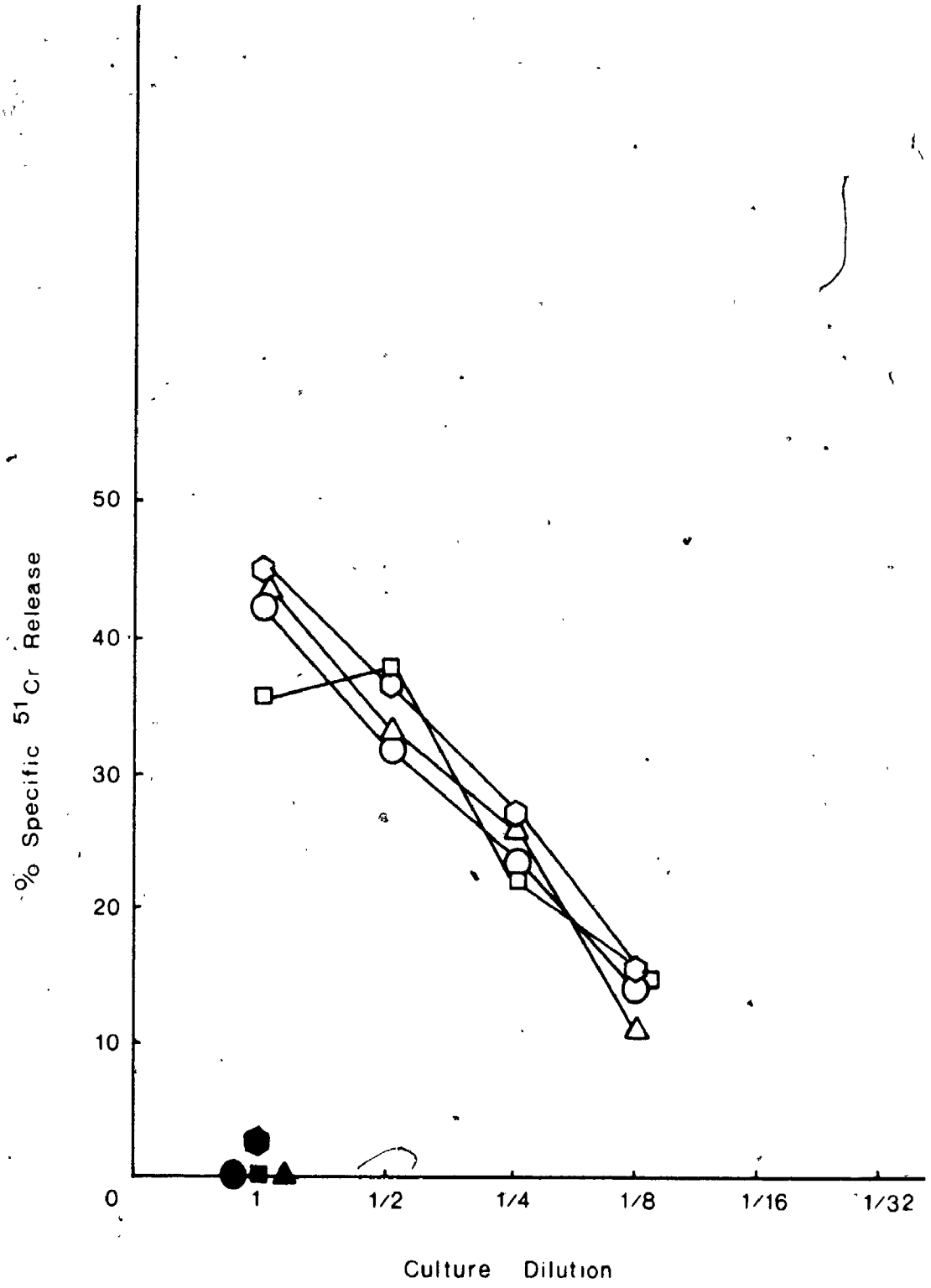

% Specific ^{51}Cr Release
50
40
30
20
10
0
1
1/2
1/4
1/8
1/16
1/32
Culture Dilution

Fig. 6

The effect of murine anti-TNP antibody on an anti-TNP modified allogeneic response

A human responder cell was sensitized to a TNP substituted allogeneic stimulator cell in the presence of normal human serum (hexagons), normal mouse serum (circles), murine anti-TNP antibody (triangles), and murine anti-TNP antibody diluted 1:100 with normal mouse serum (squares) and tested against an autologous target (closed symbols), an unmodified allogeneic target (open symbols), and the specific TNP modified allogeneic stimulating target (half symbols).

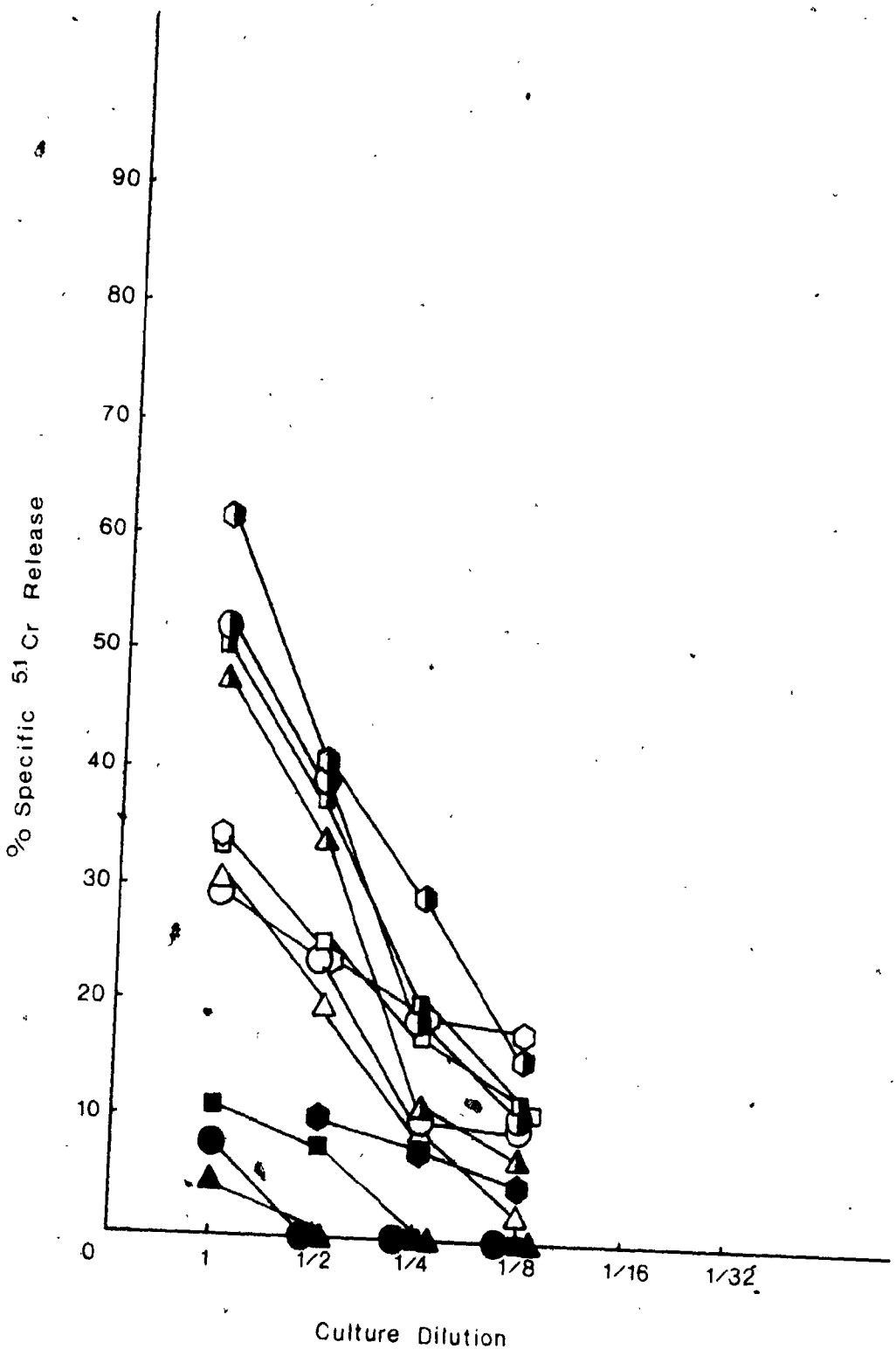
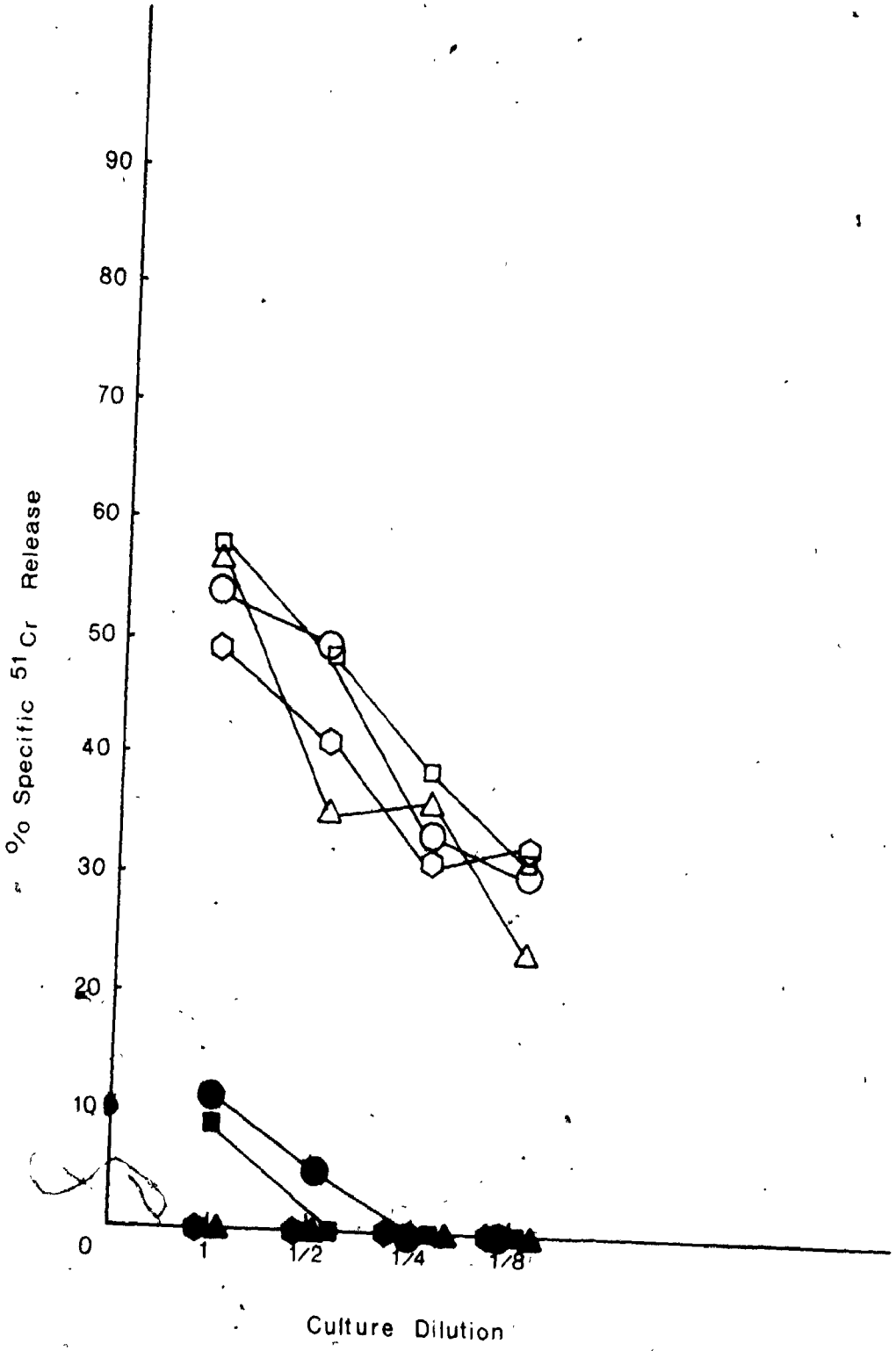

90
80
70
60
50
40
30
20
10
0
% Specific 51Cr Release
1
1/2
1/4
1/8
1/16
1/32
Culture Dilution

Fig. 7

The effect of murine anti-TNP antibody on an anti-TNP modified self response

A human responder cell was stimulated by TNP modified self in the presence of normal human serum (hexagons), normal mouse serum (circles), murine anti-TNP antibody (triangles), and murine anti-TNP antibody diluted 1:100 with NMS (squares) and tested against an unmodified autologous target (closed symbols) and a TNP substituted self target (open symbols).


90
80
70
60
50
40
30
20
10
0
% Specific ^{51}Cr Release
1
1/2
1/4
1/8
Culture Dilution

directed against a TNP modified autologous stimulator are shown. There was no activity directed at the autologous target in the presence of human serum although the presence of both the normal mouse serum and the mouse serum containing the low amounts of anti-TNP antibody did create a non-specific cytotoxic response at the lowest culture dilutions. The serum with the high anti-TNP titre did not elicit a response. The fact that the serum with the low antibody activity developed a response was due to the fact that this serum had been diluted 1:100 with pooled normal mouse serum. Any non-specific anti-self activity generated appeared to come from the pooled serum. The anti-TNP activity mounted by the CTL in response to the stimulation by a TNP modified autologous cell was quite high (50% specific ^{51}Cr release) when cultured with normal human serum. The addition of the normal mouse serum again augmented the response approximately 5-10% in the lower dilutions. Sensitizing the responders in the presence of mouse serum containing either high or low concentrations of anti-TNP antibody did not decrease the response. The anti-TNP antibodies did not inhibit sensitization to the hapten on the autologous stimulating cells.

1.6) Effects of primate anti-TNP antibody on the immunoregulation of the TNP directed autologous and allogeneic systems

The ineffectiveness of murine anti-TNP antibodies in limiting a T cell mediated response to a TNP hapten in a

human system could have been the result of species incompatibility due to structural variation between species in the Fc portion of the antibody. An examination of the effects of a more closely related antibody-T-cell system was conducted. Rhesus monkeys were repeatedly immunized with TNP-KLH over a month long period. At the end of that time serum samples were taken and tested for anti-TNP activity. Samples with a 1:512 titre were used to study antibody dependent immunoregulation of a T cell mediated anti-TNP response.

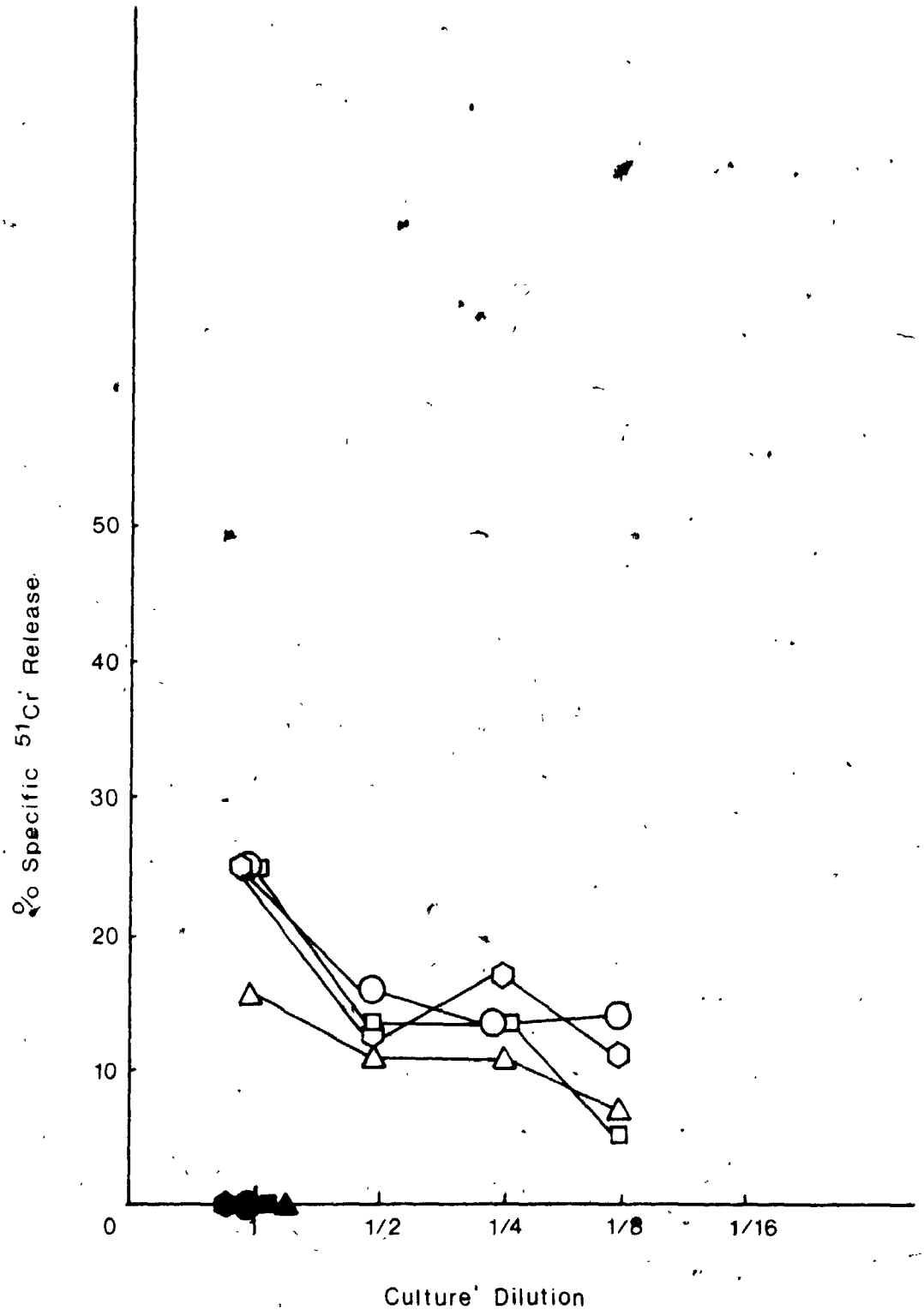
Stimulating cells were placed in Marbrook vessels and normal human serum, pooled normal monkey serum, monkey serum with a high concentration of anti-TNP antibodies, or monkey serum with a low concentration of anti-TNP antibodies added. Responders were then added to a final volume of 1 ml and a final serum concentration of 20%. The cultures were harvested on day 6, washed twice and assayed in the micro system.

Sensitization to alloantigens alone resulted in a histocompatibility antigen specific CTL. The effector did not lyse the autologous target and gave a 25% anti-B response (Fig. 8). The addition of normal monkey serum and the serum containing the low levels of anti-TNP antibodies did not change the pattern of reactivity. The primate serum with the high levels of anti-TNP antibody did affect the lowest effector cell dilution. Since there was no difference at the highest dilutions the effect was thought

Fig. 8

The effect of monkey anti-TNP antibodies on a human anti-allogeneic response.

A human responder cell was cultured with an allogeneic stimulator cell in the presence of normal human serum (hexagons), normal monkey serum (circles), simian anti-TNP antibody (triangles), and diluted simian anti-TNP antibody (squares) and then tested against an autologous target (closed symbols) and the specific allogeneic stimulating target (open symbols).


% Specific 51Cr Release
50
40
30
20
10
0
1
1/2
1/4
1/8
1/16
Culture Dilution

to be a transient phenomenon associated with that experiment. Subsequent experiments confirmed that primate anti-TNP antibodies had no effect on an anti-allogeneic response.

An effector sensitized to hapten altered allogeneic cells did not develop any anti-self reactivity. The CTL did exhibit an anti-allogeneic response that was not affected by primate serum (Fig. 9). The anti-B-TNP response was marginally higher than the anti-allogeneic indicating that the CTL recognized and responded more effectively against the modified alloantigens to which they were originally sensitized. The presence of high concentrations of primate anti-TNP antibody did not affect the degree of responsiveness (Fig. 9).

Effectors sensitized to TNP modified self did not develop any type of spontanteous autoreactivity when tested against the autologous target (Fig. 10). The pooled monkey serum and the other monkey serum samples did not possess any anti-human activity. The normal monkey serum did not affect the TNP specific response of the CTL when tested against the TNP modified self target. Primate anti-TNP antibodies added in high or low concentrations during sensitization decreased the response to altered self at some effector cell dilutions. The effect was not major and was probably due to steric hindrance of antigen recognition, not Fc dependent regulation.

Anti-TNP antibodies did not suppress the response of

Fig. 9

The effect of monkey anti-TNP antibodies on the generation of a response specific for a TNP modified allogeneic target.

A human responder cell was cultured with a TNP modified allogeneic stimulator cell in the presence of normal human serum (hexagons), normal monkey serum (circles), simian anti-TNP antibodies (triangles), simian anti-TNP antibodies diluted 1:100 with normal monkey serum (squares) and tested against the autologous target (closed symbols), an allogeneic target (open symbols), and the specific TNP modified allogeneic stimulating target cell (half symbols).

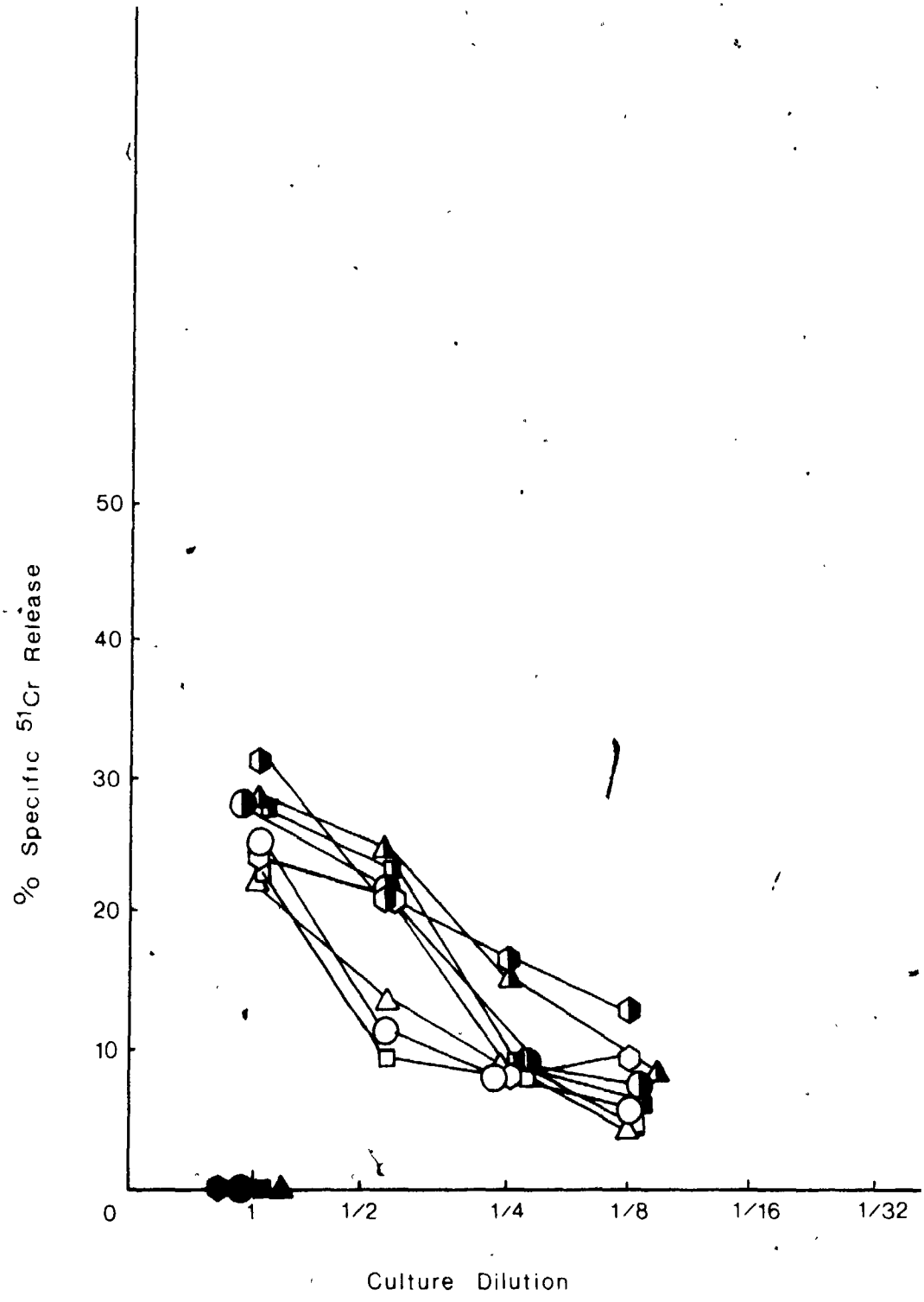
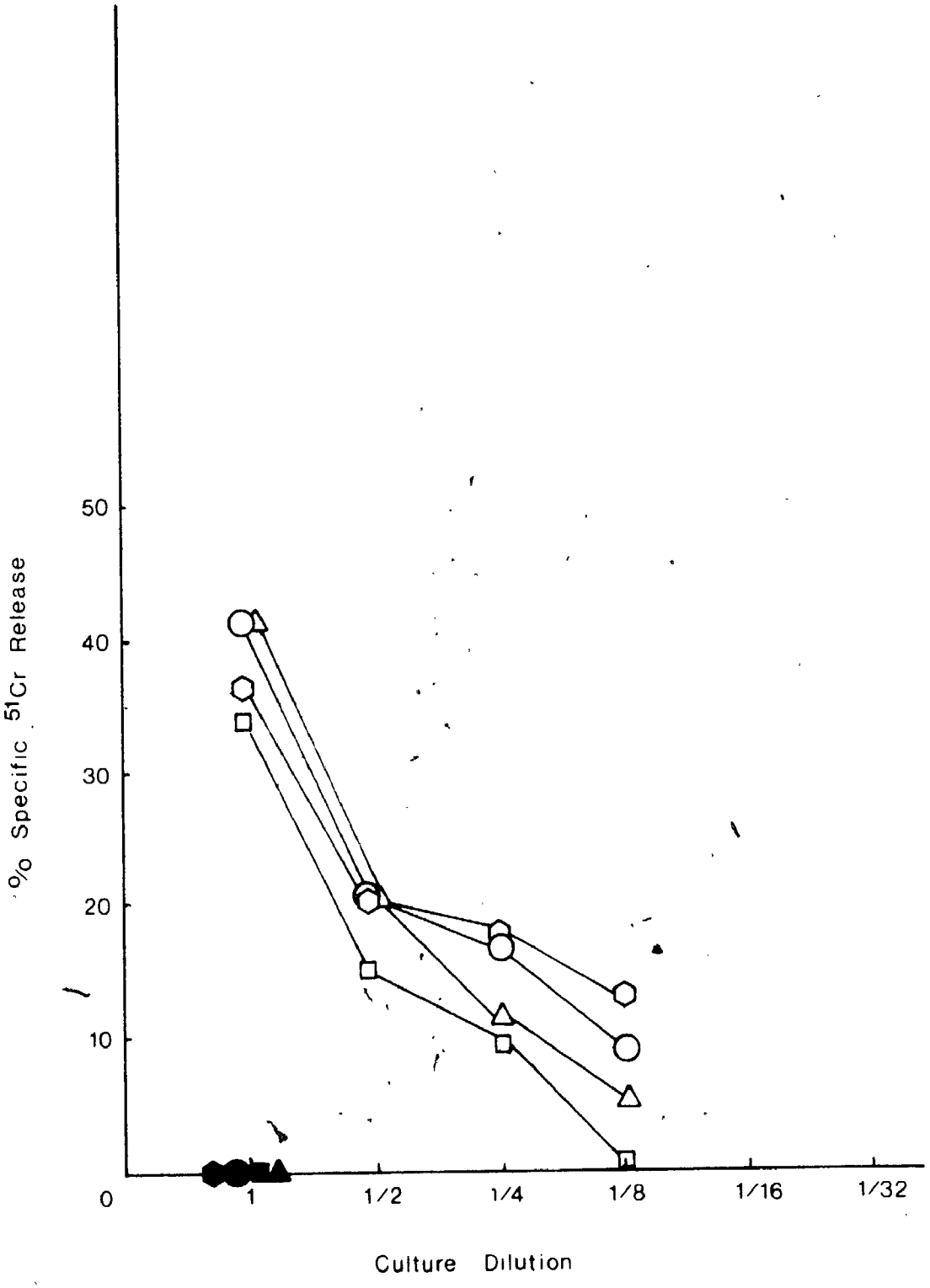

% Specific ^{51}Cr Release
50
40
30
20
10
0
1
1/2
1/4
1/8
1/16
1/32
Culture Dilution

Fig. 10

The effect of monkey anti-TNP antibody on CTL specific for TNP modified self.

A human responder cell was sensitized to a TNP modified autologous stimulator cell in the presence of normal human serum (hexagons), normal monkey serum (circles), simian anti-TNP antibodies (triangles), and simian anti-TNP antibodies diluted 1:100 with normal monkey serum (squares) and then tested against an autologous target (closed symbols) and a TNP modified autologous target (open symbols).


% Specific ^{51}Cr Release
50
40
30
20
10
0
1
1/2
1/4
1/8
1/16
1/32
Culture Dilution

CTL to hapten modified self. The same antibody could not affect an anti-allogeneic response and was incapable of suppressing the response to hapten modified alloantigens.

1.7) Effects of human anti-TNP antibody on the immunoregulation of the TNP directed autologous and allogeneic systems.

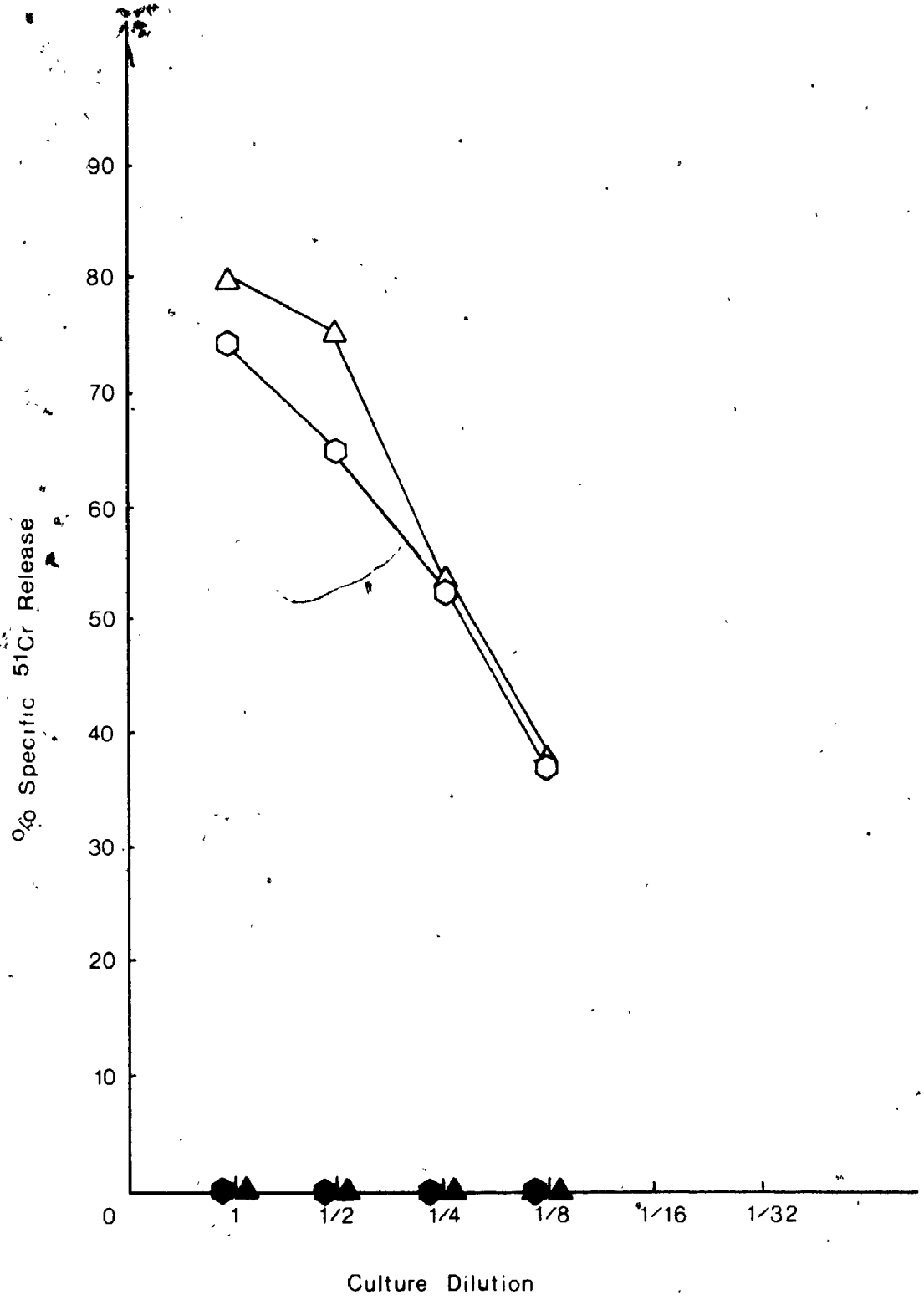
Fc dependent regulation of a CMIR did not occur across species barriers whether the species were widely disparate or very similar (see above). The only antibody dependent regulation of the response appeared to be due to masking of the sensitizing antigens during CTL generation. The effect of human anti-TNP antibody regulation on a TNP directed, T cell mediated immune response was examined. Anti-TNP antibodies were generated in a volunteer during a primary sensitization to TNP-KLH. Ten days after the administration of the antigen serum samples were collected and assayed for anti-TNP activity. Control human serum or serum containing anti-TNP activity (1:64) was added to stimulator cells in Marbrook tissue culture vessels. The responders were then added and the final serum concentration was 20%. The cultures were harvested on day 6, washed twice, and assayed in microtitre trays.

Sensitization of a CTL to alloantigens alone did not induce any autoreactivity (Fig. 11). A strong anti-B response was obtained and the addition of anti-TNP antibodies had no effect on the anti-allogeneic response (Fig. 11).

Fig. 11

The effect of human anti-TNP antibody on the generation of an anti-allogeneic response.

A human responder cell was sensitized to an allogeneic stimulator cell in the presence of pooled human serum (hexagons) and serum containing anti-TNP antibody (triangles) and tested against an autologous target cell (closed symbols) and the specific allogeneic stimulating cell target (open symbols).


90
80
70
60
50
40
30
20
10
0
% Specific 51Cr Release
1
1/2
1/4
1/8
1/16
1/32
Culture Dilution

The presentation of the hapten on an allogeneic background resulted in the appearance of autologous cytotoxicity (Fig. 12). The addition of anti-TNP antibodies did not abrogate the anti-self response. A strong allogeneic response developed that was not affected by anti-TNP antibodies. The response to the hapten on an allogeneic background was greater than the response to the alloantigens alone. The presence of the anti-TNP antibodies did not affect the CTL responsiveness (Fig. 12).

CTL directed at TNP substituted autologous stimulators were very reactive, to both the autologous target and the specific stimulating cell target (Fig. 13), in the presence of normal human serum. The cytotoxicity directed at the unmodified target may have been specific for TNP cross-reactive antigens that had developed spontaneously during the culturing of the cell. This was verified by the elimination of the anti-self response upon the addition of anti-TNP antibodies during the sensitization stage of the response. The anti-TNP antibodies also reduced the TNP specific response to 'background' levels (Fig. 13).

The antibodies were at low titres since they were obtained after a primary sensitization. The anti-TNP-allogeneic response was too vigorous to be affected by any steric effects the antibodies might create. The reduction of the vigorous anti-modified self response by the antibodies suggest that a form of immunoregulation other than antigen masking may be present.

Fig. 12

The effect of human anti-TNP antibody on the generation of a CTL specific for TNP modified allogeneic targets.

A human responder cell was sensitized to a TNP modified allogeneic stimulator in pooled human serum (hexagons) and human serum containing anti-TNP antibodies (triangles) and tested against the autologous target (closed symbols), an allogeneic target (open symbols), and the specific TNP modified allogeneic stimulator target (half symbols).

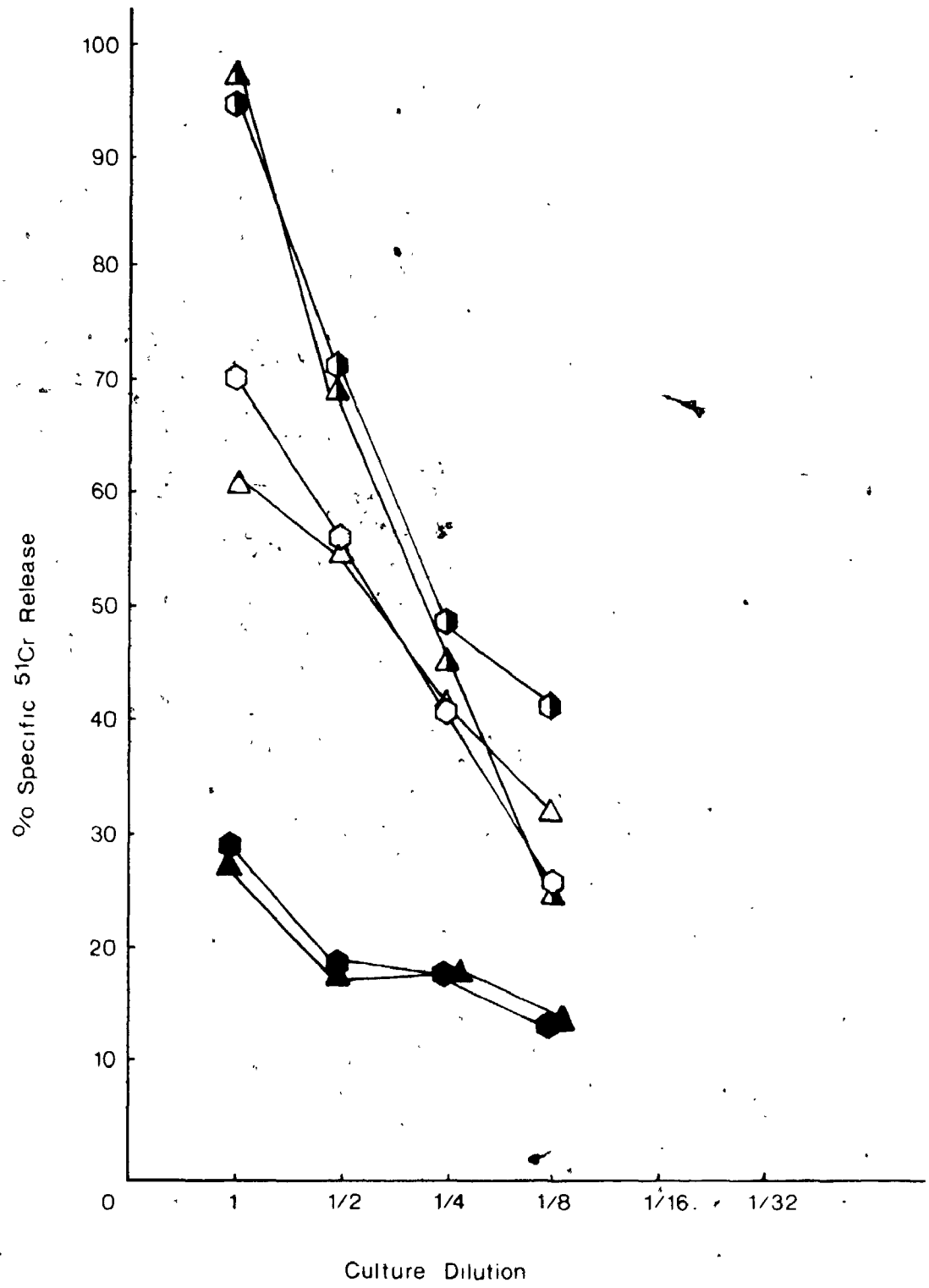
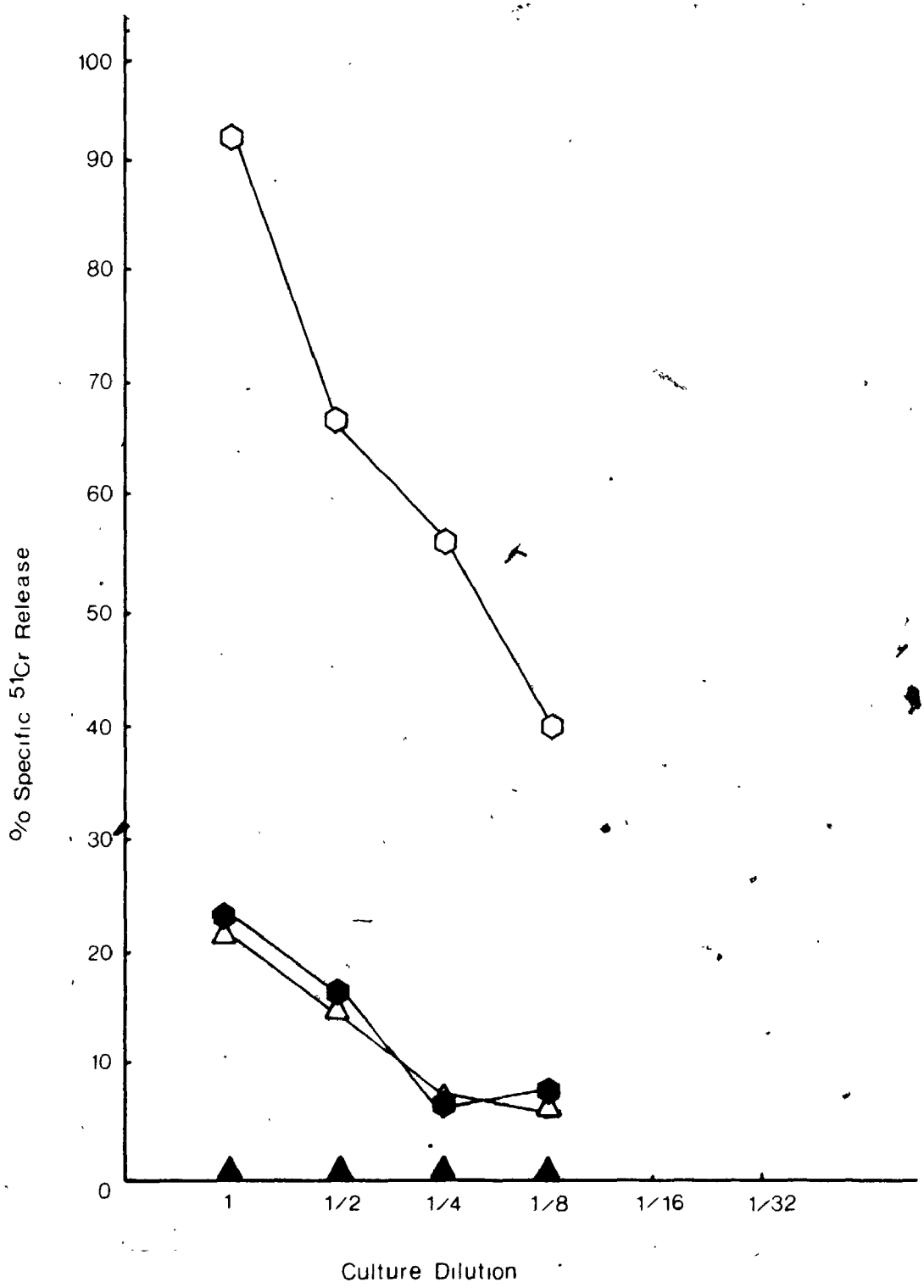

100
90
80
70
60
50
40
30
20
10
0
% Specific 51Cr Release
1
1/2
1/4
1/8
1/16
1/32
Culture Dilution

Fig. 13

The effect of human anti-TNP antibody on the CTL response to TNP substituted autologous stimulators.

A human responder cell was sensitized to TNP modified self in the presence of normal human serum (hexagons) and human serum with a positive anti-TNP titre (triangles) then assayed against the autologous target (closed symbols) and against the TNP modified self target (open symbols).


100
90
80
70
60
50
40
30
20
10
0
% Specific 51Cr Release
1
1/2
1/4
1/8
1/16
1/32
Culture Dilution

2. Characteristics of Cytotoxic Lymphocytes Specific for Naturally Occurring Non-MHC Cell Surface Antigens.

2.1) In vitro generation of CTL specific for non-MHC cell surface antigens

A number of observations have indicated that cell-mediated lymphocytotoxic (CML) activity may be directed against non-HLA targets. First, surrogate targets, chosen because of their HLA identity with donor tissue, were not suitable for transplant monitoring, whereas cells obtained from the specific donor were adequate targets (Stiller et al., 1976). Second, rejection episodes occurred in HLA identical transplants and were associated with the production of CML activity (Stiller et al., 1977). Third, in vitro sensitization of one individual's lymphocytes against another's gave rise to CML activity directed against a panel of target cells not sharing antigens in common with those of the stimulator cell (Kristensen et al., 1976a). Fourth, lymphocytes obtained from transplant recipients post-rejection were cytotoxic for target cells not sharing the same donor serological mismatches (Gluckman et al., 1977). This cross-reactive cytotoxicity was the basis for the study of cytotoxic typing lymphocytes (CTL) which could be used for the delineation of CD antigen phenotypes in a CML.

Peripheral blood was obtained from normal donors and centrifuged on a Ficoll-Hypaque gradient. The lymphocytes (PBL) were resuspended and used in the capacity as either a

responder cell, stimulator cell, or target cell. Three hundred thousand responder cells were mixed with $1x10^5$ γ-irradiated stimulator cells in a total volume of 0.2 ml and placed in flat bottom microtitre trays. After a five day incubation period at 37^oC $3x10^3$ PHA transformed, ^{51}Cr labelled target cells were added. An 8 hour CML was performed, at the end of which the trays were centrifuged and 0.1 ml of supernatant removed. The percent specific ^{51}Cr release was calculated.

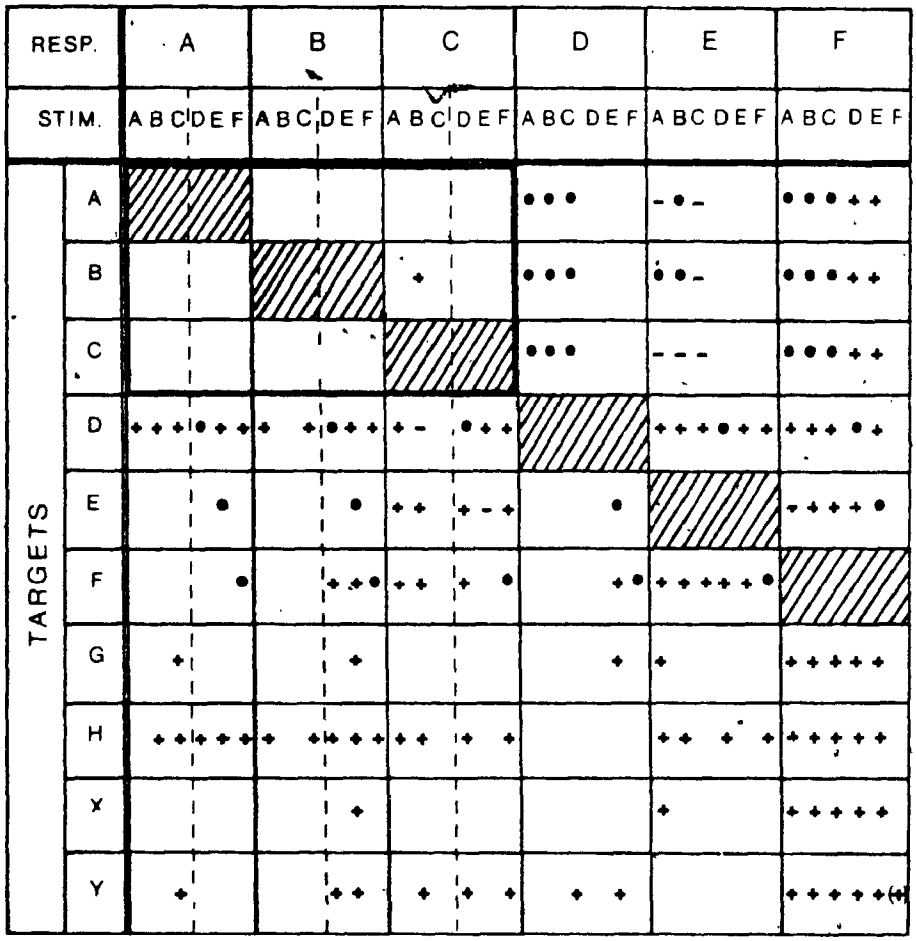
Six unrelated individuals, three HLA-A and B antigen identical, were sensitized to six stimulator cells then tested against a panel of ten target cells (Fig. 14). The degree of reactivity was divided into three groups. Less than 5% specific ^{51}Cr release was considered negative, five to ten percent was considered equivocal, and greater than 10% was considered positive. In order to define more clearly a CD antigen directed cytotoxic response all responses of less than 10% ^{51}Cr release were excluded from the analysis.

The shaded blocks represent the areas where the specific responding cells are the targets. In all the cases there were no examples of autologous killing greater than 10% (Fig. 14). Similarly culturing with the autologous stimulator failed to produce a non-specific response against the various targets in all but two of the cases. In those instances, A.Ax and E.Ex, the targets were the same (D). Target D exhibited a high degree of susceptibility to

Fig. 14

Generation of CD directed responses with a panel of normal human volunteers.

CML activities displayed by six lymphocytes with identical or very similar HLA-A and B antigens were sensitized against each other and tested against each other and targets from four other individuals. The plus signs (+) indicates reactions not explained on the basis of HLA antigens. The dot (•) indicates cytotoxicity explicable on the basis of HLA-A or B antigens. The blocks indicating autologous killing are shaded. Lymphocytes from individuals A, B, and C are HLA-A and B identical (1,2/8,12)..


RESP.
A
B
C
D
E
F
STIM.
A B C D E F
TARGETS
A
B
C
D
E
F
G
H
X
Y
A B C D
A 1,2/8,12/-,-/-,-
B 1,2/8,12/-,-/- -
C 1,2/8,12/-,-/-,-
D 1,2/8,40/3,-/6,-
E 1,2/8,21/-,-/-,-
F 1 2/8,27/2,-/-,-
G 1,2/7,17/ND/6, LDB17
H 2,3/7,15/ND/-,-
X 1,2/5,13/ND/ND
Y 1,24/15,37/ND/ ND

cytolytic effects. The black dots represent a cytotoxic response to an HLA antigen, recognized as foreign by the responding cell, that can be explained on the basis of sharing of that antigen by both the stimulator and target cell. All the responders lysed the specific stimulating cell target showing that sensitization to HLA and non-HLA antigens yielded a response in the classic manner. There were a few exceptions. Responder C sensitized to stimulator E did not lyse the E target, conversely responder E sensitized to stimulator C did not lyse target C as expected. Responder E also did not react against the A target when sensitized to A. Cross-reactive, HLA-antigen specific lysis was exhibited by responders D, E, and F. Sensitization to the antigen B-12 resulted in a response to the specific stimulating cell target but also to the third party target cells bearing the same antigens (Fig. 14). Responder E did not react to either the stimulating cell or third party targets when sensitized with C. Sensitization to B gave an anti-B response as well as a cross-reactive anti-A response, target C was not lysed. Stimulation by A gave a cross-reactive anti-B response but not a specific anti-A response, or a cross-reactive anti-C response.

The plus signs signify cytotoxic events that could not be explained on the basis of effector recognition of foreign HLA antigens, either because the target did not share any of the HLA antigens in common with the stimulator cells or the antigens shared between the two were also present on the

responder and should not have been recognized as foreign. All of the CTL tested in this system exhibited some degree of non-HLA directed cytotoxicity (Fig. 14). These cross-reactive CTL showed varying degrees of effectiveness ranging from very narrow (ie. CTL-D) to extremely broad (ie. CTL-F). Target sensitivity to this form of lysis also ranged from barely susceptible (ie. X) to very susceptible (ie. D). The specificity of these reactions, and the CTL ability to lyse one target and not another, does not change with time.

The three unrelated individuals possessing HLA-A and B antigens 1,2/8,12 produced almost no effect against each other but were cytotoxic against third party target cells (Fig. 14). This phenomenon was directed against a variety of targets expressing antigens partially or completely different from those of the responders. These particular CTL's elicited non-HLA directed cytotoxic responses when sensitized to stimulators D, E and F, that were HLA-B incompatible, and when sensitized to each other, despite complete compatibility across the HLA-A and B loci. Although no HLA-C incompatibility was detected the incidence of blanks was so high that HLA-C typing was considered uninformative. This showed that although responders A, B and C did not react against each other it was not because of lack of sensitization to foreign CD antigens. The lack of reactivity to HLA-A and B identical targets was due to a block at the efferent stage of the CML.

2.2) Inheritence of CD antigens within families

Poor correlations between serological typing and the survival of allografts between unrelated donor-recipient pairs (Halgrimson et al., 1971) as well as rejection episodes occurring between living-related, HLA-identical donor-recipient pairs (Stiller et al., 1977) indicated that the serologically detectable antigens may not be the targets for the CTL during allograft rejection. The fact that rejection episodes occurred in living-related individuals, HLA-A and B identical by serological typing, and HLA-D identical by lymphocyte typing, suggested that the CD target may not be encoded by the MHC.

To investigate the above possibilities further, CTL specific for CD antigen differences among third party target cells were generated in microtitre trays using PBL's obtained from healthy, unrelated volunteers as responder and stimulator cells. After a five day sensitization period PHA transformed, ^{51}Cr labelled target cells were added. The targets were obtained from families of sibling transplant pairs and included the donor-recipient pair, all related siblings, and the mother and father if possible. The percent specific ^{51}Cr-release was calculated after an 8 hour assay.

CTL matching with four CD specific CTL yielded cytotoxic responses not explicable on the basis of HLA antigen sharing between the stimulator and target cells (Table 8). Within

Table 8

Inheritence of CTL Targets within Family (SKER)

FAMILY MEMBER	HLA-A & B HAPLOTYPES	CTL TYPING			
		1	2	3	4
MOTHER	2-15 3-7	32	37	53	77
FATHER	1-8 1-8	12	51[1]	35	23
CHILD 1 (R)	1-8 2-15	19[1]	41[1]	24	55
CHILD 2 (D)	1-8 2-15	38[1]	39[1]	35	41
CHILD 3	1-8 3-7	20	54	42	24
CHILD 4	1-8 3-7	0	49[1]	29	0

[1] HLA-A or B sharing between the target and the stimulator cell in the CTL pair.

the family SKER CTL 1, CTL 3 and CTL 4 exhibited a quantitative difference in reactivity towards the maternal and paternal CD antigens. Since all CTL were more reactive against the maternal target than the paternal target differentiation based on target cell susceptibility to lysis cannot be excluded. The same CTL detected a quantitative difference in CD antigen identity between the MHC identical donor-recipient pair although the recipient experienced a benign clinical course. Target cell sensitivity was discounted in this case since neither the target nor the other reacted particularly well with the CTL. The remaining two siblings bearing the HLA phenotype 1,3/7,8 also showed CD antigen disparity. CTL 3 detected a quantitative difference. CD reactivity was greater with child 3 than with child 4. A qualitative difference was detected by CTL 1 and CTL 4. Both CTL gave a cytotoxic response against child 3 but were non-reactive against child 4. Lack of susceptibility to CTL activity by the child 4 target could not explain this pattern of reactivity since CTL 3 was capable of effectively lysing the target (Table 8).

CTL typing was performed on the family CROS (Table 9). The paternal MHC tissue typing could not be done, but from examination of haplotype distribution among the siblings it can be defined as 2,3/7,13. The CROS family had two sets of MHC identical siblings. The sets were mutually exclusive on the basis of HLA antigen sharing. The siblings in the first

Table 9

Inheritance of CTL Targets within Family (CROS)

FAMILY MEMBER	HLA-A & B HAPLOTYPES	CTL TYPING			
		1	2	3	4
MOTHER	1-8 2-12	9	29[1]	6	43
CHILD 1 (R)	2-13 2-12	20	70	41	65
CHILD 2 (D)	2-13 2-12	10	22	26	65
CHILD 3	2-13 2-12	28	38	38	99
CHILD 4	1-8 3-7	11	27[1]	16	7
CHILD 5	1-8 3-7	4	28[1]	12	0
CHILD 6	1-8 3-7	0	38[1]	25	0

[1] HLA-A or B sharing between the target and the stimulator cell in the CTL pair.

set containing the donor-recipient pair (2,2/12,13) were separable through CTL typing on a quantitative basis only (Table 9). This was not an accurate reflection of the CD antigen distribution since the target cell susceptibility may affect the final result of the cytolytic function. The second sibling set (1,3/7,8) showed distinctive CTL pattern differences. Child 4 was lysed by CTL 1, CTL 3 and CTL 4, child 5 was lysed by CTL 1 and CTL 3 but was not attacked by CTL 4 and child 6 was lysed only by CTL 3. The qualitative difference of reactivity vs non-reactivity clearly showed CD antigen disparity.

2.3) Effects of PHA transformation of target cells on CTL typing

It had been noted that the cross-reactivities, not explicable on the basis of known HLA-A, B or some C differences, were only observed when the cross-reacting targets had been transformed with phytohaemagglutinin (PHA). This could have indicated that the CD antigen target was a product of PHA stimulation or that, to obtain chromium release above a threshold value, the target cells had to be made into blasts. Blocking experiments were performed to determine if anti-CD antigen activity was a real phenomenon or the result of target cell manipulation with mitogens.

CTL were generated in microtitre trays and on day 5 the CML assay performed. Prior to the addition of the targets cold PHA transformed or non-transformed blocking cells were added in an attempt to block specific or cross-reactive CML

activity. The trays were incubated for 8 hours after target addition, centrifuged, 0.1 ml of supernatant was removed and the percent specific ^{51}Cr release was determined.

A responder-stimulator combination was chosen in which there was some autologous activity against the responder cell (Table 10). The addition of non-transformed autologous blockers decreased the level of the response but did not eliminate it totally, despite a blocker:target excess of 100:1. PHA transformed blockers added to the cultures effectively abrogated the anti-self response. The presence of transformed blockers impeded the ability of the CTL to function in a cytolytic capacity by diluting out the specific target antigens, while non-transformed blockers eliminated the anti-self activity by competing for the anti-self receptors on the CTL. The autologous activity was directed at antigenic determinants on the target cell surface, present as a result of PHA stimulation, and not usually found on normal, untransformed cells.

The response to the specific stimulating target was decreased by the addition of any type of blocker. Adding non-transformed autologous blockers decreased the cytotoxicity observed but not in a fashion directly related to the number of blockers. Non-transformed specific target blockers inhibited the response, particularly at the higher blocker:target ratios, indicating the cold blockers were effectively competing with the labelled targets for T-cell receptors. The addition of either PHA transformed

Table 10

PHA Effects in CTL-Typing

CELL COMBINATION	UNBLOCKED[1]	BLOCKING CELLS				
		RATIO[2]	RESP	RESP-PHA	TARGET	TARGET-PHA
ABx→A	16.0	50:1	7.4	0.0	N.A.	N.A.
		100:1	12.0	0.0	N.A.	N.A.
ABx→B	74.3	10:1	46.0	45.8	47.9	55.4
		50:1	56.2	43.2	44.0	40.5
		100:1	51.6	35.8	30.5	18.1
ABx→U	42.4	25:1	47.2	46.8	32.5	39.0
		50.1	43.7	39.5	29.2	26.4
		100:1	43.5	38.1	27.6	20.4

A anti-B cytotoxic cells were tested against the responder (A), stimulator (B), or a third party cell (U) not sharing HLA-A or B antigens with the stimulator (Bx). The blocking cells were unlabelled target cells, which, in the first group only, were the same as the responding cells.

[1] Percent specific ^{51}Cr release in the absence of blockers.

[2] Ratio of blocking to target cell concentration.

autologous blockers or PHA transformed specific target blockers inhibited the response to an even greater extent. The transformed autologous blockers at the highest ratio, decreased the response to almost the same level as the non-transformed specific target blockers. The transformed specific blockers reduced the response to 25% of the original at the highest blocker concentrations (Table 10).

Similar observation were made concerning the CD antigen directed responses. Non-transformed autologous blockers failed to inhibit the response while non-transformed specific target blockers did inhibit the response. PHA transformation increased the inhibitory effect of both blockers (Table 10). These experiments indicated that some of the antigens recognized in CTL killing are the result of PHA transformation. The CD antigen directed activity could be inhibited with either transformed or untransformed cells. This indicated that CD antigens are present on non-transformed cells and were not a product of PHA transformation. Since treatment with PHA was necessary to obtain suitable targets, the CD antigen density either increased or the target blast became more susceptible to CD activity, but the CD antigen was nevertheless present on non-transformed cells as indicated by the blocking experiment.

2.4) CTL typing of related HLA-identical transplant pairs.

CTL generated from PBL obtained from healthy, unrelated individuals had the ability to distinguish target antigen differences between MHC identical siblings (Table 8 and Table 9). The CTL could also detect differences between donor-recipient transplant pairs (Table 8), despite the fact that these pairs were MLC non-reactive and HLA-A and B identical. Although HLA-C was not tissue typed in these samples identity at HLA-A, B, and D indicated that HLA-C must be identical between the pairs, since the probability of a double crossover event occurring to give an HLA-C difference was very low. The ability of the CTL to detect CD differences between MHC identical, sibling transplant pairs, and the relationship to their respective transplant courses was explored.

CTL yielding reproducible anti-CD antigen responses against third party target cell panels were generated in the micro system using PBL's obtained from normal, unrelated donors. The CTL were tested in an eight hour CML against PHA transformed, ^{51}Cr labelled target cells from five living, related donor-recipient transplant pairs. The target cells were cryopreserved PBL taken from the donor pretransplant or from the recipient posttransplant in the course of transplant monitoring. Upon thawing, the cells exhibited >85% viability as measured by trypan-blue dye exclusion. CD antigen differences detected by the CTL were compared with the clinical course of the recipient, the

change in creatinine levels, and the number of rejection episodes occurring during the transplant course.

Five CTL detected no qualitative difference between the first donor-recipient transplant pair (TP-1) although the donor target cells were slightly more susceptible to the effects of the effector cell activity (Table 11). This particular recipient experienced only one mild rejection episode during his clinical course. Distinct qualitative differences were present in the next three pairs tested. TP-2 differs in one CTL typing while TP-3 and TP-4 differ in two CTL typings. The obvious differences in CD antigen distribution recognized by the CTL correlated with the severity of the transplant course. Each recipient experienced one or more severe rejection episodes. Two different CTL were used in the typing of TP-5 but no qualitative difference was detected. The donor target was more resistant to CTL activity than the recipient target. This recipient had a very mild transplant course with one rejection episode. There is a high degree of correlation of CTL typing with the clinical assessment (Table 11) since TP-1 and TP-5 gave the least severe rejection episodes and showed the greatest similarity on CTL matching, while TP-2, TP-3 and TP-4, different on the basis of CTL typing but not different on the basis of MHC antigen sharing, had difficult transplant courses.

Table 11

CTL Typing of Related HLA-Identical Transplant Pairs (TP)

TRANSPLANT PAIR	CTL TYPING CELLS 1	2	3	4	5	NO. OF REJECTIONS	TRANSPLANT COURSE SEVERITY	dCR
1	32.8 20.5	55.7 68.6	18.3 37.5	66.9 67.5	62.0 100.0	1	+	1.0
2	0.0 0.0	27.4 21.9	0.0 0.0	11.9 0.0	27.0 38.8	1	++++	6.9
3	0.0 16.7	22.8 28.5	0.0 0.0	0.0 0.0	0.0 13.4	2	+++	1.8
4	19.1 0.0	40.8 39.1	0.0 0.0	22.8 0.0	50.7 49.0	1	+++	3.0
5	20.0 10.0	41.0[1] 26.0	65.0[1] 65.0	70.0 22.0	— —	1	+/-	0.5

[1] Different CTL than those used in the above four donor-recipient pairs.
dCR - change in creatinine during the most severe rejection episodes

2.5) CTL typing of two recipient-one donor combinations.

CTL typing of living related transplant pairs detected CD antigen differences concommitant with an increased number of rejection episodes. The cytotoxic typing lymphocytes were tested on four two recipient-one donor combinations to determine if a correlation existed between CD discrepancies detected between unrelated donor-recipient pairs and the severity of the clinical course.

CTL were generated as previously described and tested in an eight hour CML against PHA-transformed, ^{51}Cr labelled target cells of the cadaveric donor and the two unrelated recipients. The target cells of the donor were cryopreserved spleen cells, thawed and cultured 24 hours before the addition of PHA. Recipient target cells were cryopreserved PBL taken during the course of transplant monitoring, thawed and cultured for 24 hours before the addition of PHA. The spleen cells were >75% viable and the PBL's were >85% viable on the basis of trypan blue dye exclusion.

Four CTL were used to test the compatibility of four cadaveric donors and their recipients. Donor (D) 1 shared HLA-B antigen matches with recipient (R) 1B, but did not match at any of the HLA loci with R-1A (Table 12). CTL typing showed qualitative CD differences between D1 and R-1B. R-1B had two moderately severe rejection episodes but retained his allograft. D1 and R-1A had similar CTL cytotoxic patterns even though R-1A experienced a very

Table 12

CTL Typing of Two-Recipient One-Donor Combinations

	HLA-A	HLA-B	HLA-D	CTL TYPING CELLS 1	2	3	4	NO. OF REJECTIONS	COURSE SEVERITY	dCR
RECIPIENT 1A	2,26	21,40	-,-	12	22	51	54	1	4+	?
RECIPIENT 1B	28,11	8,12	-,-	0	20[1]	0	7	2	2+	1.3
DONOR 1	1,29	8,12	1,6	11	25[1]	18	46			
RECIPIENT 2A	2,30	8,60	3,-	0	38	17	0	2	+/-	2.2
RECIPIENT 2B	24,29	12,60	B17,-	16	16[1]	17	74	2	3+	1.7
DONOR 2	1,2	7,17	B17,6	0	15[1]	0	100			
RECIPIENT 3A	2,-	18,21	B17,-	11	18	40	56	1	2+	1.5
RECIPIENT 3B	1,28	8,60	3,6	0	39	43	61	3	4+	8.8
DONOR 3	2,-	14,27	6,-	12	33	19	78			
RECIPIENT 4A	28,3	18,59	6,-	11	28	11	50	1	4+	6.5
RECIPIENT 4B	2,29	12,27	B17,-	18	12	6	66	2	4+	6.5
DONOR 4	2,32	12,-	-,-	15	29	39	59			

[1] HLA-A or B sharing with the stimulator cell in the CTL pair.
dCR - change in creatinine during the most severe rejection episodes.

severe rejection episode and underwent a nephrectomy on day 12. D2 differed from R-2A by two CTL and from R-2B by two CTL. Both recipients had two rejection episodes although the R-2A episodes were very mild and the R-2B episodes severe. Neither recipient lost their allograft. D3 was CD similar to R-3A and different in one CTL typing from R-3B. R-3A experienced one rejection episode and after successful treatment retained his allograft. R-3B experienced three rejection episodes, a very severe clinical course, and was subsequently nephrectomized on day 99. Both R-4A and R-4B experienced severe clinical courses and rejection episodes, and underwent nephrectomies on day 25 and day 67, respectively. Comparison of CTL typing with D4 did not show a qualitative difference in CD antigen distribution (Table 12). Thus there was no correlation between CD antigen similarity of donor and recipient pairs and transplant courses in this series.

2.6) Generation of CTL using pooled stimulators.

Rapid detection of MHC incompatibility is essential to the success of allotransplantation between a qualified recipient and a cadaveric donor. Sheehy et al. (1975) developed a system in which a responder cell was primed in vitro to recognize a specific HLA-D antigen. This effector was then stored and would respond, specifically and rapidly, when restimulated by cells of a person containing the same HLA-D haplotype as the original stimulating cell. This

'primed typing lymphocyte' could be used in a rapid detection proliferative assay to distinguish specific HLA-D antigens within twenty-four hours.

A variation of this method was proposed by Segall and Bach (1976) in which a pool of lymphocytes were used to stimulate the responder instead of a single stimulating cell. Activation of cells of a given responder by cells of a given stimulator results in the proliferation of a subpopulation of responder cells. Activation of the same responder by another population of stimulator cells results in the proliferation of another different, or partially overlapping, subpopulation of responder cells. The pooling of stimulating cells from increasing numbers of individuals should result in an increased level of subpopulation activation among the responders until a plateau of response is reached. At the plateau, all responding cells capable of being activated by histocompatibility antigens have been activated and the plateau is independent of the stimulating cells used in the pool (Segall and Bach, 1976). CTL generated using pooled stimulator cells could also be used to detect MHC compatibility between donor-recipient pairs. The antigens sensitized against would include not only the HLA antigens but the CD antigens as well.

Four million irradiated stimulator cells of one type or $4x10^6$ cells from a pool consisting of five different stimulating cells in equal proportions were mixed with $4x10^6$ responder cells in a Marbrook culture to a total

volume of 1 ml. The cultures were incubated for 6 days in a primary MLC. The effectors were harvested, washed and assayed in microtitre trays against PHA-transformed, ^{51}Cr labelled autologous, allogeneic, or pooled allogeneic target cells in a four hour CML. The percent specific ^{51}Cr release was determined.

The effector could mount a response against all of the individuals used in the pool when sensitized by that individual stimulator and tested against the specific target (Table 13). The pool sensitized effector was capable of recognizing both the pool and the individual members of the pool. The amount of activity was the same as that observed when the responder was sensitized by the individual stimulators. Pooled sensitization resulted in the development of some autologous reactivity. This reactivity was present in subsequent experiments and did not increase when the length of the assay was increased from four to eight hours (Ward and Jevnikar, unpublished observation) suggesting that the activity observed was not a specific anti-self reactivity. Specificity for the individual members of the pool was retained after the pooled sensitization even though the effectors were sensitized to a broad band of histocompatibility antigens.

Although pools of five stimulators retained their specificities for the individual members of the pool, there has been evidence to suggest that a minimum pool size of from ten to twenty stimulators was needed to approach, or be

Table 13

Effect of Pooled and Individual Stimulators on CML[1]

Individual Stimulator				Pooled Stimulator			
STIMULATOR	EFFECTOR CONC.[2]	TARGET	% ^{51}Cr RELEASE	STIMULATOR	EFFECTOR CONC.	TARGET	% ^{51}Cr RELEASE
A	8	A	31.7	P[3]	8	A	26.8
	4		23.0		4		22.9
	2		20.5		2		17.4
B	8	B	21.0	P	8	B	26.8
	4		22.0		4		28.4
	2		14.7		2		20.1
C	8	C	21.6	P	8	C	27.6
	4		13.3		4		19.8
	2		14.1		2		14.9
D	8	D	24.0	P	8	D	25.[illegible]
	4		21.4		4		24[illegible]
	2		15.7		2		19.3
E	8	E	34.7	P	8	E	32.5
	4		28.6		4		23.3
	2		15.4		2		18.8
P	8	P	31.3	P	8	R	14.3
	4		28.3		4		-
	2		20.9		2		-

1 Responder is the same in both groups.
2 Responder x 10^6.
3 P = pooled stimulator.

on, a plateau of stimulation that was as high as, or higher, than the highest stimulation by any of the individual cells in the pool (Segall and Bach, 1976). Transplant recipients that had experienced relatively benign to moderately severe clinical courses were used as responders in a CD typing assay to determine the feasibility of using pooled stimulator cells to sensitize potential transplant recipient cells. These cells could then be stored and used in rapid detection assays depending on the availability of donors.

Cryopreserved pretransplant PBL's from three individuals were used as responder cells and sensitized in the microtitre system to donor spleen cells, a pool of five randomly selected, unrelated individuals or a pool of twenty randomly selected, unrelated individuals. After five days the effectors were tested in an eight hour CML against PHA transformed, ^{51}Cr labelled autologous, donor, and pooled target cells. The percent specific ^{51}Cr release was determined.

Responder SCA had a benign clinical course without any rejection episodes, and was well matched serologically with the donor (Table 14). Sensitization to the donor and then testing against the specific stimulating target failed to elicit a response. SCA could not recognize any foreign histocompatibility antigens on the donor. Sensitization to P1 resulted in a percent specific release of 100 against the specific stimulating target and the development of a response against the donor target (Table 14). Similarly

Table 14

Effects of Pooled Stimulators on Sensitization of Pretransplant Cells.

RESPONDER	STIMULATOR	TARGETS			TRANS.COURSE SEVERITY
		RESPONDER	DONOR	POOL	
SCA	DONOR	0.0	0.0	-	
	POOL1[1]	0.0	69.6	100.0	+/-
	POOL2[2]	0.0	72.6	57.0	
EPP	DONOR	0.0	0.0	-	
	POOL1	0.0	58.9	80.7	+/-
	POOL2	0.0	40.0	53.7	
CRO	DONOR	0.0	0.0	-	
	POOL1	0.0	0.0[3]	47.6	++
	POOL2	0.0	1.2[3]	32.1	

[1] Pool of five unrelated individuals
[2] Pool of twenty unrelated individuals
[3] Not significant

RESPONDER	HLA-TYPING	DONOR HLA-TYPING
SCA	2,-/5,18	2,-/5,13
EPP	1,2/18,-	28,-/50,-
CRO	1,2/27,37	1,10/8,16

sensitization to P2 gave a specific stimulator response of 57% and a higher response against the donor target.

Responder EPP also had a very benign transplant course despite the fact there were not any serological matches between him and his donor (Table 14). In vitro sensitization to the donor failed to elicit an anti-donor response yet sensitization to either P1 or P2 resulted in both the specific anti-stimulator response as well as an anti-donor response (Table 14).

Responder CRO had had two unsuccessful transplants prior to her third. There was serological matching with the third donor at one of the HLA-A loci (Table 14) and prior sensitization to one of the donor histocompatibility antigens (unpublished observation). The transplant course was benign initially but on day 62 her creatinine level began to rise. Despite immunosuppressive therapy her creatinine level continued to rise and remained high throughout the rest of her clinical course. The allograft was subsequently removed. Sensitization to the donor failed to elicit an anti-donor response. Sensitization to either the five member pool or the twenty member pool gave a cytotoxic response to the specific pooled targets that was 50% less than the responses developed by the other recipients' effector cells. This particular effector also failed to develop any activity against the donor target when stimulated by the pools. The ineffective generation of a response in this circumstance may be due to the

immunosuppressive regime the patient was on prior to the allotransplantation.

The use of primed CTL's in the matching of prospective transplant donor-recipients does not give a clear picture of the subsequent clinical course that may be expected by the patient. The response magnitude does not correlate with the graft outcome. Treatment of individuals with immunosuppressive drugs may influence the ability of the PBL's from that individual to react in an MLC-CML. There has also been evidence to suggest that lymphocytes from uremic individuals are less able to provide a strong in vitro response following in vitro priming and cryopreservation (Sondel et al., 1982). The variation in the ability of cadaveric tissue to stimulate in an MLC could also affect the magnitude of the in vitro response generated in a CML. Spleen cells are usually better stimulators than PBL's but in some instances they can be non-stimulatory (unpublished observation, Sondel et al., 1982).

2.7) Analysis of MHC-CD interactions in the general population

Recognition of histocompatibility antigens at both the afferent and efferent stages of the CML is necessary for the lysis of allogeneic target cells (Miggiano et al., 1972). Although matching of the classical HLA-A and B histocompatibility antigens prior to human allograft transplantation has been correlated with increased graft

survival there has been evidence to suggest that these antigens may not be as important as originally thought and may be influenced by other factors, including distribution of antigens (Baldwin et al.,.1980), host factors and centre variation (Ting, 1981), and cytolytically defined target antigens (Bach et al., 1972).

CTL specific for CD antigens can be generated using allogeneic responder-stimulator cell combinations. The ability of the CTL to recognize the CD antigens may be affected by the sensitization of the effector to the classical MHC antigens. In some cases it appears that the expression of cytotoxicity is enhanced by LD and SD disparity between the responder and the stimulator (Bach et al., 1976), but in other cases the CD specific activity can be inhibited by the addition of a more powerful MHC allogeneic stimulator cell (Sondel and Bach, 1976). There is also evidence that the recognition of minor histocompatibility antigens in the murine system is MHC restricted (Bevan, 1977). The influence of the MHC during the sensitization of CTL to CD antigens may play a role in the ultimate acceptance or rejection of an allograft post-transplantation.

Peripheral blood was taken from two different panels of normal donors. The lymphocytes were removed using Ficoll-Hypaque centrifugation, resuspended, and used in the capacity as either a responder cell, a stimulator cell, or a target cell. The two panels differed in that the members of

Panel 1 were disparate at their HLA-A and B alleles and the members of Panel 2 were identical at both of the HLA-A alleles and identical at one or both of the HLA-B alleles. Typing of the HLA-C locus was incomplete and considered uninformative. Three hundred thousand responder cells were mixed with $1x10^5$ γ-irradiated stimulator cells in a total volume of 0.2 ml and placed in flat bottom microtitre plates. After a five day incubation period PHA-transformed, ^{51}Cr labelled target cells were added. An eight hour CML was performed and the percent specific ^{51}Cr release was calculated.

The number of total positive cytotoxic events in both matrices were compared in a 2 x 2 contingency table (Table 15). Of the total of 680 reactions 259 of them were positive (39%). Both panels had approximately the same number of positive reactions and the same number of negative reactions. There was no significant difference in the distribution of the positive and negative reactions between the two panels. CD antigen specific reactions have been previously defined as cytotoxic events that could not be explained on the basis of effector recognition of foreign HLA antigens, either because the target did not share any antigens in common with the stimulator cell used in the sensitization or the antigens shared between the two were also present on the responder and should not have been recognized as foreign. An analysis of 'CD antigen' directed activity vs antigen sharing was performed (Table 16).

Table 15

A 2 x 2 Contingency Table Analyzing the Effect of Antigen Sharing on a Positive Cytotoxic Response

	Positive[1]	Negative	Total
Panel 1	125	195	320
Panel 2	134	226	360
Total	259	421	680

$X^2 = 0.193$

$v = 1$

$p > 0.5$

[1] - Numbers of positive cytotoxic events within the panel

Table 16

A 2 x 2 Contingency Table Analyzing the Effect of Antigen Sharing on CD Antigen Directed Responses

	CD Directed[1]	Negative[2]	Total
Panel 1	55	195	250
Panel 2	98	226	324
Total	153	421	574

$X^2 = 5.827$

$v = 1$

$p < 0.025$

[1] - Number of CD reactions within the panels

[2] - Number of negative reactions within the panels

Panel 2 had a significantly higher number of CD directed reactions (32%) than Panel 1 (21%).

Panel 1 was used to determine the effect of HLA-A and B antigens on the generation of cytotoxic responses in a system where 44% of the positive reactions were classed as CD antigen directed. The total positive reactions were segregated based on HLA-A and B antigen sharing between i) responder and stimulator, ii) responder and target, and iii) stimulator and target. These reactions were then compared, using contingency tables, with the remaining positive reactions. Responder-stimulator sharing was not a prerequisite for the generation of a cytotoxic response in this system (Table 17). There was a significant inverse correlation ($p<0.005$) with antigen sharing between responder and target (Table 18). The classic model of histocompatibility antigen interaction would suggest that antigen sharing between the stimulator and target was a prerequisite for the development of a cytotoxic response. Analysis of stimulator-target shared antigens in this system showed that there was no significant correlation ($p>0.1$) between antigen sharing and reactivity (Table 19).

In this study and similar studies (Fig. 16) effectors sensitized to a specific stimulator did not always lyse targets with the same degree of effectiveness. Targets without any histocompatibility antigens in common with the stimulator could be lysed to a greater extent than targets totally histocompatible with Sx. This is not consistent

Table 17

A 2 x 2 Contingency Table Analyzing the Effect of Responder-Stimulator Sharing within a Matrix

	Positive[1]	Negative[2]	Total
Sharing	74	126	200
No Sharing	51	69	120
Total	125	195	320

$X^2 = 0.442$

$v = 1$

$p > 0.25$

[1] - Number of positive cytotoxic events within Panel 1

[2] - Number of negative cytotoxic events within Panel 1

Table 18

A 2 x 2 Contingency Table Analyzing the Effect of Responder-Target Sharing within a Matrix

	Positive[1]	Negative[2]	Total
Sharing	51	113	164
No Sharing	74	82	156
Total	125	195	320

$X^2 = 7.888$

$v = 1$

$p < 0.005$

[1] - Number of positive cytotoxic events within Panel 1

[2] - Number of negative cytotoxic events within Panel 1

Table 19

A 2 x 2 Contingency Table Analyzing the Effect of Stimulator-Target Sharing within a Matrix

	Positive[1]	Negative[2]	Total
Sharing	71	93	164
No Sharing	54	102	156
Total	125	195	320

$X^2 = 2.071$

$v = 1$

$p > 0.1$

[1] - Number of positive cytotoxic events within Panel 1

[2] - Number of negative cytotoxic events within Panel 1

with the current concepts of effector cell specificity. Target cell susceptibility may play a role in these apparently anomalous observations. In addition, although emphasis had been placed on the specific reactivity of an effector cell a strong indiscriminant effect has been reported (Kuichi and Takasugi, 1976). To eliminate the effects of differential target cell susceptibility, and to distinguish between specific and non-specific cytotoxic reactivity, a statistical method, termed Interaction Analysis, was used to re-evaluate the data. A review of the procedure is outlined in the Materials and Methods

After performing the analysis the number of significant positive reactions was reduced from 39% of the total reactions to 19%. The instances of a target being lysed by most of the effectors, regardless of the stimulator, were not apparent in the matrix. Antigen sharing between the responder and the stimulator was not necessary for the generation of a response (Table 20). There was a significant association ($p<0.025$) between the requirement for responder-target antigen sharing and the number of positive responses that were devehowever (Table 21). A highly significant association ($p<0.005$) was observed between stimulator-target antigen sharing and the incidence of positive cytotoxic reactions (Table 22).

In contrast to the low correlation observed in this situation prior to the interaction analysis, antigen sharing between the stimulator and target resulted in the

Table 20

A 2 x 2 Contingency Table Analyzing the Effect of Responder-Stimulator Sharing within a Matrix After Interaction Analysis

	Positive[1]	Negative[2]	Total
Sharing	36	164	200
No Sharing	25	95	120
Total	61	259	320

$X^2 = 0.137$

$v = 1$

$p > 0.50$

[1] - Number of positive cytotoxic events within Panel 1

[2] - Number of negative cytotoxic events within Panel 1

Table 21

A 2 x 2 Contingency Table Analyzing the Effect of Responder-Target Sharing within a Matrix After Interaction Analysis

	Positive[1]	Negative[2]	Total
Sharing	22	142	164
No Sharing	39	117	156
Total	61	259	320

$X^2 = 5.921$

$v = 1$

$p < 0.025$

[1] - Number of positive cytotoxic events within Panel 1

[2] - Number of negative cytotoxic events within Panel 1

Table 22

A 2 x 2 Contingency Table Analyzing the Effect of Stimulator-Target Sharing within a Matrix After Interaction Analysis

	Positive[1]	Negative[2]	Total
Sharing	43	121	164
No Sharing	18	138	156
Total	61	259	320

$X^2 = 9.738$

$v = 1$

$p < 0.005$

[1] - Number of positive cytotoxic events within Panel 1

[2] - Number of negative cytotoxic events within Panel 1

development of a greater number of positive cytotoxic responses. This suggested that the classic MHC directed responses segregated within the selective portion of the lysis. The significantly positive responses within the selective and non-selective portions were separated into those that could be defined as directed at MHC antigenic determinants and those that were CD directed (Table 23). There was a highly significant correlation ($p<0.001$) between the selective-MHC directed responses and the non-selective-CD directed responses. The MHC directed responses were detected predominantly in the selective compartment of the interaction analysis while the CD directed responses resided primarily within the non-selective portion (Table 23).

Table 23

A 2 x 2 Contingency Table Analyzing the Distribution of MHC and CD Directed Responses within an Interaction Analysis

	Selective[1]	Non-selective[2]	Total
MHC Directed	41	41	82
CD Directed	20	69	89
Total	61	110	171

$X^2 = 14.023$

$v = 1$

$p < 0.001$

[1] - Number of selective cytotoxic events within Panel 1

[2] - Number of non-selective cytotoxic events within Panel 1

DISCUSSION

1. Characteristics of Cytotoxic Lymphocytes Directed against Haptenic Antigens on the Surface of Autologous and Allogeneic Cells

The destruction of altered autologous cells by CTL in the human system may be the most important biological role of the immune system. The surveillance phenomenon has been studied extensively in the murine system with the development of numerous *in vivo* and *in vitro* models examining lysis of syngeneic cells modified by virus (Zinkernagel and Doherty, 1974a), chemicals (Shearer, 1974), and tumour associated antigens (Wagner and Rollinghoff, 1973). The same techniques have been adapted for the study of T cell mediated altered self cytolysis in man (Freidman et al., 1978; Shaw et al., 1978). In these reports TNP modified autologous stimulators alone produced either a poor or non-existant proliferative or cytotoxic response after six days of primary sensitization.

Freidman and his group sensitized in microtitre trays and found that TNP specific CTL were only generated in cultures that contained both TNP derivatized autologous stimulators and an additional signal, either allogeneic cells or soluble antigen to which the donor had been previously sensitized (Freidman et al., 1978). These effectors were specific for TNP modified autologous cells and did not react with unmodified autologous cells or TNP

modified allogeneic target cells. Shaw performed his sensitization in tissue culture flasks and found that there was minimal reactivity directed at the TNP modified autologous target cells following the primary sensitization. A secondary sensitization with stimulator cells more heavily derivatized with TNP was necessary to allow the development of the response (Shaw et al., 1978). The CTL were capable of lysing modified autologous cells and modified unrelated human target cells.

In our system, using Marbrook tissue culture vessels for the six day sensitization procedure, sensitization to TNP modified self resulted in the development of an anti-TNP modified self response at all effector to stimulator ratios (Table 1). The maximum effector-stimulator contact, for optimum sensitization to derivatized autologous antigens, occured when there are $4x10^6$ responders and the same number of stimulators in the inner chamber. CTL sensitized to modified autologous cells were also capable of recognizing modified allogeneic cells (Table 2) at the same optimum ratio. In contrast to the anti-modified self response, this response was restricted to the optimum responder-stimulator ratios only. The sensitization ratio of 4:4 is identical to the optimum ratio determined by Fradelizi and Dausset (1975) during the maximization of the Marbrook system for the use with human CTL directed against allogeneic targets.

An anti-TNP response was observed regardless of the degree of derivatization of the stimulator and levelled off

when the cells were substituted with 10 mM TNBS (Figs. 2 and 3). At higher concentrations of the hapten modifier greater derivatization did not increase the response. There may be a correlation between the extent of TNP derivatization and the ability of the cells to act as stimulators, similar to that observed in the murine system by Forman et al. (1977b). Further substitution either does not affect the sites recognized by the CTL or at approximately 10 mM TNBS all sites available for substitution are filled and higher concentrations of hapten have no effect.

Using the Marbrook system we were able to generate CTL capable of lysing hapten modified autologous and hapten modified allogeneic target cells. This was accomplished without the addition of allogeneic cells or soluble antigens. Furthermore the effectors were able to produce a cytolytic response after a primary sensitization. The use of the micro system by Freidman in the sensitization stage of the response may dictate the need for `helper' factors. Our system, in contrast to Shaw's, was able to produce a response after a primary sensitization. During the primary MLC Shaw and coworkers sensitized with a stimulator substituted with 3 mM TNBS. Restimulating cells and targets were substituted with 10 mM TNBS. Both our stimulating cells and target cells were substituted with 10 mM TNBS and yielded optimum results. Our results confirm the findings of Forman et al. (1977b) in the murine system. Target cells recognized by an effector require the same degree of

haptenic substitution as the stimulator used in the initial sensitization. The Shaw group appeared to obtain a poor primary response since the stimulating cell and the target cell did not have the same amount of hapten derivatization. They did generate a response during the secondary stimulation when they used an Sx substituted to the same degree as the targets.

The ability of CTL to recognize 'altered self' antigens suggests that they play an important role in immunological surveillance. Effectors sensitized to chemically altered autologous cells exhibit cross-reactivity with allelic variants of self MHC (Burakoff et al., 1976; Shaw et al., 1978). The investigation of several different effectors sensitized to altered self showed that all reactivity was specific for the hapten-MHC antigen complex and no reactivity was directed against the unmodified targets by the unseparated effector populations (Tables 3-5).

It has been suggested that the identity of the cell that mediates the hapten directed cytotoxicity is a T cell (Shaw et al., 1978). Fc depletion of the cultures had no effect on the ability of the cells to elicit an anti-TNP modified self response. Separation of the cells into SERF+ (T) and SERF- (non-T) fractions resulted in a decrease in responsiveness in the T depleted fraction and no change in the T cell fraction (Shaw et al., 1978). After a primary sensitization in the Marbrook system separation of the effectors into their respective SERF+ and SERF- fractions

affects the level of cytotoxicity (Tables 3-5). Reactivity in the SERF+ (T) fraction against the specific stimulating cell target either remains the same as in the unfractionated effector group (Table 5) or decreases (Table 3 and Table 4). The SERF- fraction exhibited higher levels of cytolytic activity than the unseparated populations in all cases tested. The increased reactivity was observed when tested against modified self targets and when tested against modified allogeneic cells.

The predominance of reactivity in the non-T cell fraction and the increased level of responsiveness after separation suggests that one of the major components of anti-TNP activity is a non-T cell. Naturally occurring cytotoxic cells composed of heterogenous and discrete subclasses of non-T effector cells (ie. NK) have been implicated as the primary line of defense during the preservation of the structural integrity of an organism. These cells exist in high frequencies in unstimulated hosts (Roder and Haliotis, 1980) and do not display classical immunological memory (Greenberg and Greene, 1976; Stutman, 1981).

Although the natural killer cells are thought to be different from T cells, evidence is accumulating that the majority of the cells in the NK subset, though heterogenous for surface markers, belong to the T cell subset (Kaplan and Calleweart, 1978). Rabbit anti-T antisera plus C' abrogates NK activity. The lytic C' was necessary since antibodies

alone did not affect the reactivity (Kaplan and Calleweart, 1978). Evidence has been obtained that the cell involved in the response against modified self are $OKT38^+$ (McMichael et al., 1982). Removal of E-rosettes by Ficoll-Hypaque separation enriches for NK cells. T cells with high affinity E receptors are the least active in NK populations (Klein, 1981).

The cells obtained in our SERF- fractions were large granular lymphocytes, while the cells in the SERF+ fraction were small to medium lymphocytes. Morphologic studies of human NK cells show that they are large granular lymphocytes of the T cell lineage (Seksela and Timonen, 1980). The effector cells in the SERF- fraction that are involved in the anti-TNP cytolytic response may be from the NK class of cell. Removal of the SERF+ (T) cell augments the response further than would be expected through the concentration of the effector cells (Tables 3-5). This implies that the T cells are functioning in an active capacity to inhibit the cytolytic ability of the SERF- effector cell population. A parallel has been observed in the murine system where suppressor T lymphocytes specific for tumour antigen arise one day after exposure of recipient mice to syngeneic tumour (Fujimoto et al., 1976).

Shaw found that most of the anti-TNP reactivity resided in the SERF+ fraction of the separation while we found that the majority of the reactivity was in the SERF- fraction. The majority of fresh NK cells in PBL are capable of forming

E rosettes under optimum conditions and thus appear to have a low affinity receptor for SRBC (Ortaldo and Herberman, 1980; Klein, 1981). After 7 day in vitro culturing a small, but distinct, subset of E rosette forming cells can be found in the NK cell fraction (Ortaldo and Herberman, 1980). The reactivity observed in our SERF+ fractions could be due to this E receptor shift within the NK cells. Shaw and coworkers used a secondary stimulation for the generation of their effector cells and found that the predominant anti-TNP activity resided in the E rosette forming cell fraction. The possibility that a further shift with time may result in more NK cells developing high affinity E receptors cannot be excluded.

Helper T cells can potentiate the generation of an effector cell through the release of helper factor (Watson et al., 1980). These factors can be obtained from human peripheral blood T lymphocytes during allogeneic interaction and activation by soluble antigen. There has been evidence to suggest that human Ia-like MHC determinants play a role in the elaboration of helper factors and that these factors can aid in the generation of TNP specific CTL (Freidman et al., 1979). Recent reports have shown that helper factors derived from an allogeneic response can augment NK activity and facilitate long term culturing of activated cells with NK characteristics (Henney et al., 1981; Kuribayashi et al., 1981). Interleukin 2 (IL-2) has been identified as one factor that increases NK cell activity and it is believed

that the enhancing effect of interferon functions through increasing the density of the IL-2 receptors on the effector cell surface thereby augmenting the effect of the factor (Kuribayashi et al., 1981).

The addition of allogeneic culture supernatants to non-responder and poor responder CTL elicited an anti-TNP response from both the autologously sensitized CTL and the modified self stimulated CTL (Figs. 3 and 4). The action of the CTL were specific for modified self targets. There was no reactivity against the unmodified self target or the allogeneic target used in the supernatant generation. The reactivity of the TNP modified self sensitized effector increased slightly under the influence of the supernatants and the autologously stimulated responder developed a cytolytic response capable of lysing TNP modified autologous targets.

Sensitization of responder cells to modified self results in the generation of large granular lymphocytes that reside in the SERF- fraction. These lymphocytes acquire E receptors with continued culturing and are regulated by a SERF+ suppressor cell. Activation of the effector cells can be accomplished with IL-2 derived from allogeneic culture supernatants in the absence of antigenic stimulus. The effector thus generated is specific for altered self.

Responder cells placed in culture with irradiated modified self are activated and T helper cells produce factors that promote the development of a T/NK cell

effector, this effector may be suppressed or inactivated by a T cell within the SERF+ fraction. The anti-TNP modified self system provides a model in which the effector target is a small, stable antigenic determinant in conjunction with a broad MHC background. Using this model we studied the effect of the addition of murine, simian, and human anti-TNP antibody on the immunoregulation of the anti-allogeneic response.

The use of TNP as the stimulator antigen for both the antibody and the effector cells in the response provided us with an antigen that could not be altered to a state unrecognizable by antibody, so that the formation of a tripartite complex between antigen, antibody and T cells (Sinclair, 1979) could take place. Within this complex the interaction with the antigen involves an antigen receptor while the interaction with the antibody takes place via an Fc receptor on the surface of the immunocompetent cell (Sinclair and Chan, 1971). Presumably the effector cell would be inactivated by an Fc dependent signal and cytolytic activity directed at the target cell would be abrogated.

The addition of murine anti-TNP antibodies to any of our assay systems did not affect the cytolytic response regardless of the stimulator for the responder cells (Figs. 5-7). Similarly the addition of simian anti-TNP antibody had no effect on the generation of cytotoxic reactivity (Figs.

8-10). Human anti-TNP antibodies derived from a primary sensitization to TNP-KLH did not affect an anti-allogeneic response (Fig. 11) or an anti-TNP-allogeneic response (Fig. 12). The presence of the antibody did reduce the response directed at a modified self target to background levels (Fig. 13).

The failure of the murine and simian antibodies to feedback inhibit the allogeneic response could have been due to the structural variation between species in the Fc portion of the antibody. Mouse γ chain subclasses differ from each other more than human γ chain subclasses (Fudenberg et al., 1978). It has been noted that γ-chains having a common evolutionary origin may be shared by closely related species. Although immunologic cross-reactivity has been observed between human immunoglobulin subclasses and gorilla and chimpanzee IgG subclasses (Fudenberg et al., 1978) the Fc portion of the Rhesus monkey antibody used in these experiments may have been sufficiently dissimilar to the human Fc portion of antibody to not allow feedback inhibition to take place.

In order for an Fc-mediated antigen-antibody complex inactivation of an immunocompetent cell to take place the antibody must interact with the cell, binding the same antigen, through a cytophilic attachment of the Fc portion of the antibody (Sinclair et al., 1976c). Approximately 20-25% of T lymphocytes in human peripheral blood have Fc receptors for IgG (Reinherz et al., 1980), 60-70% have

receptors for IgM (Reinherz et al., 1980), while the rest have no receptors (Lung and Singal, 1981). During an in vitro MLC the number of cells bearing Fc receptors for IgG increases (Moretta et al., 1981) and activation also involves an increase in the Fc receptor density (Yoshida and Anersson, 1972).

The NK function of the T cell subsets has been localized in the IgG Fc receptor bearing population (Kaplan and Calleweart, 1978; Moretta and Fauci, 1980; Pross and Baines, 1980) although Fc receptor negative NK cells can be produced under certain conditions (Kay et al., 1979). The effector cells within our system should have been capable of receiving an inhibitory signal from an anti-TNP IgG antibody. Human anti-TNP antibody induced a suppressive effect on modified self sensitized effector cell (Fig. 13) but not against the response directed against the modified alloantigens (Fig. 12). Abrogation of the cytolytic effect in the one instance (Fig. 13) indicates that the antibodies could play some role in regulating the response. The degree of suppression in the presence of such low titred (1:64) antibodies suggests that Fc dependent feedback may be the form of immunoregulation rather than a simpler mechanism, such as antigen masking. Furthermore during allogeneic reactions T cells release non-specific stimulatory factors that can induce resistance to the feedback suppressive effect of the antibody without affecting the immunosuppressive effects of antigen masking (Sinclair et al., 1976c). The absence of

any kind of inhibitory effect during sensitization to TNP modified allogeneic cells may be the result of the allogeneic effect factors present.

2. Characteristics of Cytotoxic Lymphocytes Specific for Naturally Occurring Non-MHC Cell Surface Antigens

Observations by several different laboratories have indicated that the classical HLA antigens may not be the target for effector CTL. DBA/2 mastocytoma cells stripped of serologically reactive H-2 antigens were susceptible to cytolysis by CTL (Edidin and Henney, 1973), there are poor correlations between serological typing and the survival of allografts between unrelated human donor-recipient transplant pairs (Halgrimson et al., 1971), rejection episodes occurred in related HLA identical transplants and were associated with the production of CTL (Stiller et al., 1977) and CTL generated between HLA identical unrelated individuals exhibited a high degree of cytotoxicity against the stimulating cell target (Schapira and Jeannet, 1974). These experiments indicate that CTL recognize antigens other than the HLA-A, B, C (and D), or that they recognize antigens in a different way than either antibody or homozygous typing cells. This recognitive ability occurs in vivo and in vitro.

It is possible to generate CD antigen specific CTL that yield reproducible cytolysis on allogeneic lymphocyte targets without detectable HLA-A, B, and C sharing between

the stimulator and target cells (Fig. 14). In the early work of Kristensen et al. (1976b), 12 CTL were tested in parallel against lymphocytes from a random population sample. Based on a pairwise analysis of those CTL, 11 of them could be classified into two groups of significantly correlated CTL. A larger panel of 19 CTL was subsequently tested against 100 unrelated randomly chosen target cells (Kristensen, 1978). Pairwise analysis of the larger panel indicated that the order of the individual CTL could be arranged in such a way to suggest the existence of at least three different group specificities. These groups exhibited positive associations, were loosely defined and could contain several specificities. A considerable number of negative associations were detected that suggested that the traits defined by the CTL may be genetically governed by alleles at one locus (Kristensen, 1978).

Generation of CD antigen specific responses can be accomplished by using normal, unrelated volunteers as responders, stimulators and targets (Fig. 14). In all of the panels we tested, approximately 35% of the reactions could be classed as positive. CD specific cytotoxicity is defined as a positive reaction not explicable on the basis of recognizable MHC antigen sharing between the responder, stimulator and target cells involved in the reaction. The reactions classed as CD directed comprised 25% of the total interactions or 71% of the total positive reactions (unpublished observation). This concurs with the

observation by Mawas et al. (1974) that 30% of the allogeneic responder-stimulator combinations tested against target cells totally MHC distinct from the stimulator gave a positive response.

The comparison of two panels; one with a high degree of HLA-A and B antigen sharing between the cells involved in the cytolytic reactions and the other with minimal sharing, indicated that the MHC antigens did not have any effect on the generation of a cytolytic response (Table 15). A significantly higher ($p<0.025$) number of CD specific cytolytic reactions were observed in the panel with the greater MHC sharing (Table 16). This observation suggests that some form of restriction is involved in the recognition of the CD target antigen, similar to that seen in the response of cytolytic T cells against either chemically modified or virus infected target cells (Shearer et al., 1976b; Zinkernagel and Doherty, 1974a). Within these systems the target cell must share some MHC genes with the original stimulating cell used in the induction of the response. The requirement for a similar MHC background on which to recognize a foreign antigen suggest that CTL recognize altered self and that recognition of CD antigens may parallel the systems mentioned above.

Further analysis of the requirement for MHC antigen sharing between the various cells involved in generating a positive cytotoxic response was carried out. The responses were separated into groups based on antigen sharing between

two of the three cells involved and then tested in 2x2 contingency tables. On the basis of these analyses it was apparent that in the in vitro generated CTL system there was no requirement for MHC antigen sharing in the generation of a positive cytolytic response (Tables 17-19). Since half of the positive reactions detected within the panel examined were CD directed, and comparison of similar and dissimilar panels showed that a HLA-A and B similarity enhanced the generation of the CD specific response (Table 16), it was expected that a greater number of positive responses would be observed when there was stimulator-target antigen sharing. There was a tendency towards the development of more positive responses when there was antigen sharing but it was not significant ($0.25>p>0.10$) (Table 19).

The determination of positive cytotoxic events in the above analysis was dependent on the observed percent specific ^{51}Cr release. The possibility of effects other than the cytotoxic potential of the effector cells causing the death of the target cells has to be considered. These effects include specific immunological effects, such as sensitization to other target cell antigens, and nonspecific factors, such as cell crowding or poor preparation of target cells. In order to look at the specific reactivity exhibited by the effector cells, we split the percent specific ^{51}Cr release values into representative 'selective' and 'non-selective' cytotoxicity using a method termed Interaction Analysis (Takasugi and Mickey, 1976).

Selective cytotoxicity has been defined as that part of the cytotoxic reaction caused by a special interaction between the effector and the target cell. Non-selective cytotoxicity is the reactivity generated by a particular effector against all target cells. The differences in reactivity in this form of cytotoxicity are due to differences in the sensitivity of each target cell to lysis. Some target cells are more susceptible to lysis from all effectors than others, while some targets are very resistant (Fig. 14).

The recognition that effector cells differ in their cytotoxic capabilities and that target cells differ in their sensitivity makes it imperative that studies be tested in a two dimensional matrix varying effector and target cells. Interaction analysis predicts the cytotoxic reactivity based on the mean reactivity of each effector, the mean sensitivity of each target and the overall average. From these values each cytolytic reaction is analyzed and the part of the reaction that does not reflect a special relationship is noted. Any variation from this non-selective reactivity is scored as selective.

Contingency table analysis of the specific reactivity of the panel showed a strong association ($p<0.005$) between stimulator-target sharing and the development of a positive cytotoxic event (Table 22). The total number of positive reactions was reduced to 50% of the pre-interaction analysis value. The fact that half of the positive reactions

cells of cancer patients. Until an analysis of the cross-reactive cytolytic responses of the two systems is made it is impossible to state whether any or all of the effectors are capable of recognizing antigens partially identical or common to both sets of target cells.

The similarity of the effector cell subtypes and target recognition patterns among the three systems suggest that there is one common pathway for the expression of these forms of cell mediated immune responsiveness. Pre-NK cells, once activated, acquire E and Fcγ receptors and become mature NK cells (Sekela and Timonen, 1980). These cells have T cell antigens (Kaplan and Calleweart, 1978) but are less differentiated than normal T cells and may represent some form of pre-T cell (Sekela and Timonen, 1980). The $T^{+}E^{-}$ NK cell acquire E rosette receptors after exposure to thymosin, a thymic hormone (Kaplan and Calleweart, 1980). Kaplan and Calleweart (1980) have defined NK cells as pre-thymic, committed T cell precursors comprised of multiple clones of monospecific cells expressing receptors for antigens which in their pattern of distribution closely resemble self and non-self histocompatibility antigens.

Ortaldo and Herberman (1980) have proposed a model to explain the relationship between NK cells and T cells where NK cells belong to a pre-T cell subclass and develop along the main pathway of differentiation to mature T cells. This relationship may also represent the expression of cytotoxic activity in most cell mediated immune responses. The

indicates that despite the expression of NK-like activity there are specific cytolytic T cells present in the mixture. The in vitro CML assay system could be measuring all T cell mediated responses against foreign histocompatibility antigens and that the system, as previously defined, involves several different effector cell types.

The non-selective cytotoxic response does not lyse targets indiscriminately and so may be responding to a broader specificity present on all target cells. These reactions may also be a result of a polyspecific effect directed at different antigens on each of the targets. The cold target inhibition study indicates that the target of an MHC antigen independent response is present on both PHA transformed and non-transformed cells (Table 10). If the non-selective portion of the response were truly non-specific then the addition of any unlabelled competitor cell would interfere with the expression of cytolytic activity regardless of the MHC antigen distribution.

Addition of the specific target blocker to a culture containing an effector eliciting a CD directed response decreased the response while the addition of the responder blocker, which should share the common carrier antigenic determinants, had no effect on the activity (Table 10). Addition of the responder blockers to the anti-allogeneic effector did inhibit the cytotoxicity although the specific target blocker had a more pronounced effect. This indicates that the CD directed, non-selective responses have some

specificity. The specific stimulating target blocks more effectively than other blockers suggesting that the effectors are recognizing an antigen unique to that target, but other third party blockers can partially inhibit the response (unpublished observation), indicating that there are broad specificities shared by all cells that can be recognized by a sensitized effector.

Although non-selective responses exhibit a degree of specific reactivity, as determined from blocking experiments, it is difficult to distinguish these effects from such non-specific effects as target cell sensitivity in the normal CML response. In family studies this becomes more apparent. Clear cut differences between MHC identical siblings can be demonstrated through CTL typing (Tables 8 and 9). Child 3 and Child 4 in the family SKER exhibit unidirectional differences that could be attributed to a greater sensitivity to lysis by the targets of Child 4 (Table 8). Examination of the cytolytic patterns expressed by the CTL between Child 1 and Child 2 show that the differences in cytotoxic activity directed at the two targets could not be due to the effects of target cells sensitivity alone since both targets can be preferentially lysed by certain CTL (Table 8). The response of the CTL are not directed at known, shared MHC antigens and probably represent the class of response directed at the non-MHC (CD), specific target that could be detected in the cold target inhibition experiment (Table 10). CTL reactivity can be

placed into three main groups; i). MHC directed and specific, ii) CD directed and specific, and iii) non-selective and non-specific.

The three forms of reactivity are overlapping and additive in the CML assay and may be responsible for the inability of CTL to distinguish and match CD antigen identical individuals during cadaveric cross-matching (Table 12). Living related transplant pairs that are MHC identical can be more easily compared for CD antigen sharing (Table 11). Transplant pairs 1 and 5 show a mild form of target cell differential sensitivity yet the recipient in each of these pairs experienced very mild clinical courses. The other donor-recipient pairs had distinct CD differences that were not related to target cell sensitivity and that correlated with the clinical course of the recipient. Some CTL were capable of killing both targets equally well while others could only kill one target (Transplant Pair 4). In some instances a CTL was capable of killing one of the pair efficiently (Transplant Pair 2, CTL 4) and another CTL could lyse the other member of the pair more efficiently (Transplant Pair 2, CTL 5). This suggests that the target that is being recognized is specific and definable through CTL typing.

As stated above the <u>in vitro</u> generated CTL appears to exhibit three types of cytolytic activity. The selective, specific reactivity includes cytolytic activity against MHC antigens (Table 23) and any reactivity directed at cell

surface antigens is specific in the classical sense. There must be MHC antigen sharing between the stimulator and the target in order to realize the full cytotoxic potential of the CTL. This form of cytotoxicity is probably the result of cytotoxic T lymphocyte reactivity. The non-selective, specific reactivity is not MHC restricted and appears to be mediated by an NK-like cell. The exact subset of T/NK cell that this particular effector belongs to could be determined through the use of monoclonal antibodies specific for T or NK cell surface markers. This activity constitutes approximately 50% of the positive responses generated within the assays and may represent the CD directed activity observed frequently in in vitro assays. It appears to play a role in vivo as well. MHC identical, related transplant pairs show a correlation between the expression of this form of cytotoxicity and the severity of the clinical course. The final form of reactivity, the non-selective, non-specific cytotoxicity, is largely dependent on the target cell sensitivity. This activity is not related to histocompatibility sharing and may only be separated from MHC antigen specific responses through interaction analysis.

CONCLUSIONS

During our investigation of the response against haptenated target cells we found that the major portion of the cytotoxic activity resided in the SERF- subset of the effectors and consisted primarily of large, granular lymphocytes. The lack of E receptors and the morphology of the cell involved suggest that the cell mediating the response could belong to a subset of cells termed Natural Killer (NK) or Natural Cell Mediated Cytotoxicity (NCMC) cells (Seksela and Timonen, 1980; Klein, 1981; Stutman, 1981). Similarly, studies of the killing patterns of in vitro sensitized CTL directed at allogeneic target cells indicated that a major component of the CD directed cytotoxic response may be of NK cell origin by virtue of its lack of classical MHC restriction and the segregation of the responses through interaction analysis.

Studies have demonstrated that MHC restriction of T cell mediated cytotoxicity is necessary in some instances, indicating that cytotoxic T cells recognize foreign antigens in association with self determinants (Dickmeiss et al., 1977; McMichael et al., 1977). Lack of apparent MHC restriction in both of our systems indicate that self recognition may not be required for the development of a cytotoxic response, however, in situations where T cells are recognizing either alloantigens or haptenated self, portions

of the MHC molecule must be shared and can serve as self. Other parts of the molecule, whether they are the distinguishing alloantigen or the hapten, are recognized as foreign and can be construed as non-self. Within this context the feature distinguishing self from non-self is that the former is shared between the responder and the stimulator during a response while the latter is not. Further evidence for the ubiquity of self and non-self in all systems is suggested by the cross-reactivity observed between alloantigens and conventional antigens (Bevan, 1977; Finberg et al., 1978). Bevan showed that murine CTL capable of killing targets with minor H-2 differences usually required that the target have the same major H-2 antigens as the responder. Within the effector population there developed a subset of cells that were able to lyse allogeneic targets with the same minor H-2 antigens as the responder. In the latter paper effectors sensitized to virus infected self could not only lyse the altered self target but were capable of lysing allogeneic targets.

In both the systems above the non-shared determinants are recognized in the context of the shared determinants. Similar observations have been made by Shaw and Shearer (1978) with regards to the cytotoxic response to TNP modified autologous cells. Using cold target inhibition experiments their results demonstrated that the determinants recognized by the effectors could be placed into two groups, a broadly defined common determinant and a more specific

determinant not widely shared by unrelated humans (Shaw and Shearer, 1978). There has been ample evidence showing that specific MHC determinants, including HLA-A, B, C, and D, can act as the target antigen in cytotoxic responses against unmodified target cells (Kristensen et al., 1975; Eijsvoogel et al., 1976; Grunnet et al., 1976; Ball and Stastney, 1982). Broader specificities have also been detected, using cytotoxic lymphocytes to tissue type large populations, that do not define monospecific traits and have been postulated to represent the carrier part of the antigenic determinant (Kristensen et al., 1976b).

Our observations indicate that the effector involved in the recognition of TNP modified self after a primary sensitization is an NK-like cell. Recognition of the CD determinants during an in vitro CML also appears to be mediated by an NK-like cell, while recognition of MHC antigens is mediated by a more conventional T cell type. Activated NK cells can lyse a broad range of allogeneic cells and effector-target HLA A and B differences are not a pre-requisite for activation (Pross and Baines, 1980). Certain target cells are lysed more readily than others by NK cells indicating that target cell susceptibility to lysis is important in the expression of NK mediated cytotoxicity. NK cells are also capable of lysing the K562 tumour target cell, which is MHC negative, suggesting that the MHC antigens may not be the target for NK reactivity (Pross and Baines, 1980). The cross-reactivity exhibited by the NK

cells indicates that the target antigens involved in the response must be widely shared by the population.

Our examination of the CD antigen directed CTL showed that a portion of the non-selective cytotoxicity was observed to be specific for definable, non-MHC antigens (Table 23). Greenberg and Takasugi (1980) have made similar observations during the delineation of the NK reactivity towards tumour targets. Non-selective results, such as target susceptibility, were eliminated through the use of interaction analysis. Cold target inhibition experiments revealed the existence of a polyspecific system that had the appearance of non-selectivity in direct testing. Further analysis revealed significant positive correlations between groups of target cells and tentatively identified the TA1, TA2, and TA3 target specific antigens (Greenberg and Takasugi, 1980).

All three systems, the anti-tumour antigen, the anti-alloantigen, and the anti-modified histocompatibility antigen, show reactivity against a broad, common antigen and specific reactivity against a more polymorphic determinant (Greenberg and Takasugi, 1980; Kristensen et al., 1976b; Shaw and Shearer, 1978). Within two of these systems three broad specificities have been defined using almost identical techniques. Kristensen (1978) has found that CD reactivity defines three major target antigens within the general population and Greenberg and Takasugi (1980) have defined three target antigens that can be recognized by the effector

cells of cancer patients. Until an analysis of the cross-reactive cytolytic responses of the two systems is made it is impossible to state whether any or all of the effectors are capable of recognizing antigens partially identical or common to both sets of target cells.

The similarity of the effector cell subtypes and target recognition patterns among the three systems suggest that there is one common pathway for the expression of these forms of cell mediated immune responsiveness. Pre-NK cells, once activated, acquire E and Fcγ receptors and become mature NK cells (Sekela and Timonen, 1980). These cells have T cell antigens (Kaplan and Calleweart, 1978) but are less differentiated than normal T cells and may represent some form of pre-T cell (Sekela and Timonen, 1980). The T^+E^- NK cell acquire E rosette receptors after exposure to thymosin, a thymic hormone (Kaplan and Calleweart, 1980). Kaplan and Calleweart (1980) have defined NK cells as pre-thymic, committed T cell precursors comprised of multiple clones of monospecific cells expressing receptors for antigens which in their pattern of distribution closely resemble self and non-self histocompatibility antigens.

Ortaldo and Herberman (1980) have proposed a model to explain the relationship between NK cells and T cells where NK cells belong to a pre-T cell subclass and develop along the main pathway of differentiation to mature T cells. This relationship may also represent the expression of cytotoxic activity in most cell mediated immune responses. The

expression of activity may be characteristic of the stage of the T cell development and would become more specialized as maturation continued. Taken collectively our data and that of the others outlined above suggest that cell mediated responses against foreign antigens revolve around the recognition of a self (common) and a non-self (polymorphic) determinant. The recognition of the broader specificities appears to reside in the NK cell subset while the more polymorphic determinants are specifically recognized by the functionally mature T cells. Elucidation of the particular target antigen involved in a CMIR is complicated by the number of effector cell subtypes present and the particular portion of the antigen that they are recognizing.

Early recognition of an antigen is carried out by an NK/pre-T cell. In instances where there is a high degree of homology with self, such as in TNP modified systems or between living-related sibling transplants, NK cell maturation and lytic activity are expressed. Some degree of specialized activity develops as the effectors mature further. Recognition of alloantigens proceeds as above but because of the greater heterogeneity (a higher degree of non-self) more mature, functionally specialized cytotoxic T cells are either recruited or develop from existing responder cell populations. These CTL recognize distinct, larger antigenic determinants and because of this increased recognitive ability are able to express an increased cytolytic function. In cases where there is a high degree

of self but also a high degree of non-self (ie. in Panel 2, Table 16) a greater number of CD directed responses will develop.

The above model does not take into account that in response to some stimuli mature T cells may differentiate into activated NK cells, although there has been evidence to suggest that such a transition is possible (Ortaldo and Herberman, 1980). It does offer a possible explanation for the difficulty that has arisen in delineating the specific target antigens in the various CMIR. The degree of 'selfness' of the target dictates the level of activation or recruitment that will develop. This in turn decides which effector cell type will be activated, though it appears that more heterogenous targets activate more effector subtypes, and which determinant will be the target of the cytolytic activity.

SUMMARY

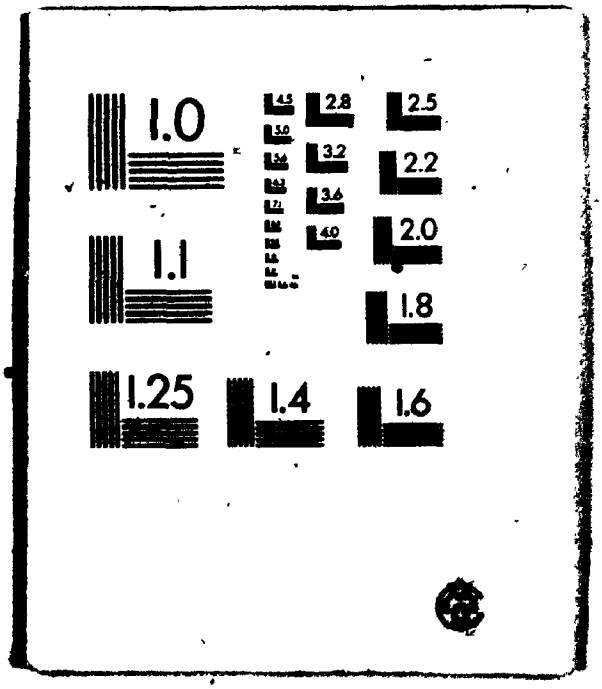
CD directed responses can be defined in the simplest way possible as the observed positive cytotoxic responses that are not explainable using the conventional MHC antigens as targets for the reaction. Two systems, using chemically modified targets and allogeneic targets, were studied.

Sensitization to modified self resulted in the development of effectors capable of lysing modified allogeneic target cells (Tables 3-7), thus MHC restriction was not observed. The major portion of the cytotoxicity resided in the SERF- fraction (Tables 3-7). The cytotoxicity could also be developed, without the specific sensitizing antigen, in the presence of factors derived during the generation of an allogeneic response (Figs. 3-4). On the basis of the cell morphology (a large granular lymphocyte), the development of a response in the presence of an IL-2 like factor, and its residence in the SERF- fraction, it was concluded that the predominant effector cell within this system was an NK-like cell.

CTL generated using randomly selected responder, stimulator, and target cells exhibited varying patterns of lysis depending partially on the effector reactivity and the target susceptibility (Fig. 14). The cytotoxicity could either be CD directed (ie. no MHC target antigens) or MHC specific (Fig. 14). Some CD directed responses displayed specificity in their reactivity. These CTL were capable of

lysing one target preferentially over another while a second CTL would exhibit reactivity against the second target and not the first (Tables 8-9, Fig. 14). This suggests that target cell susceptibility is not the only form of CD expressed reactivity. Similarly, cold blocking experiments indicate that the CD directed cytotoxic response has a specific component that can be inhibited only by the specific target cell blocker (Table 10). Other third party cells could partially inhibit the response (unpublished observation). The broad degree of lysis in some cases depended on the level of effector reactivity and the target cell susceptibility. Taken together there appear to be three forms of reactivity present; 1) MHC explainable 2) CD directed that is recognizing specific antigens on the surface of the cell that are not the standard MHC antigens (Tables 8 and 9, Fig. 14) and 3) CD reactivity that is not specific for a particular cell surface antigen but is due to target susceptibility (Target D, Fig.14) and/or effector reactivity (Effector F, Fig.14) and may be based on the genetic makeup of the particular cell involved, on the mode of preparation and handling of the cells, or the assay conditions. The first two forms of reactivity are stable in that a particular effector can retain its specificity for a given CD or MHC target antigen for several years after the initial studies.

The sharing of MHC antigens between responder, stimulator, and target cells has no effect on the generation



of cytotoxic responses (Table 15), but the generation of CD directed responses is more likely when there is greater MHC antigen sharing between those cells (Table 16). Specific CD reactivity could be observed against MHC identical transplant pairs (Table 11). This reactivity is not based on target susceptibility or effector reactivity since the various CTL have differential lysing capability. The target antigens present on the surface of these cells, within the pair, are specific and distinct from the MHC antigens. During the clinical course of the recipients some instances of severe anti-donor responses developed. The specific CD cytotoxicity is assigned more frequently when MHC antigen sharing is greatest and may represent both an in vivo and in vitro response against modified self.

An analysis of the effect of MHC antigens on the generation of cytotoxic responses indicates that there is no MHC restriction at the HLA-A and B loci between responder-stimulator (Table 17) and responder-target (Table 18) pairs. There is also no significant correlation between positive cytotoxicity and MHC antigen sharing between the stimulator and the target cells (Table 19). The apparent lack of restriction suggest that the cell involved in this response may be an NK-like cell. Dissection of the CTL responsiveness into selective and non-selective cytotoxicity (Takasugi and Mickey, 1976) showed the appearance of MHC directed cytotoxicity in the selective portion of the response (Table 22), but again there was no MHC restriction

(Tables 20 and 21). The selective cytotoxicity may represent the MHC directed, classically T cell mediated, response since there is a requirement for MHC antigen sharing between the stimulator and target (Table 22). The lack of MHC restriction could be attributed to an incomplete MHC typing scheme used in the analysis. All other cytotoxicity (ie. non-selective) would contain the CD directed cytotoxic response and may be mediated by an NK-like cell.

Within both the hapten modified target antigen system and the allogeneic target antigen system a similar sequence of events may be occurring. The responding cell comes in contact with the `foreign' stimulating cell. There is a primary reaction against modified self components of the cell carried out in a non-restricted fashion by an NK-like cell. If no other major antigenic differences are detected (ie. MHC alloantigens) then the main form of cytotoxicity is mediated by this cell type with a minor shift in the development of more specific effector cells. This form of reactivity is the specific CD reactivity and is observed between MHC identical transplant pairs, certain randomly selected individuals from a normal population, and in response to hapten or tumour antigen altered self. This cytotoxicity is observed as reactivity generated against broad, common (ie. self) determinants in both the haptenated (Shaw and Shearer, 1978) and alloantigen (Kristensen et al., 1976b) systems.

If other, greater antigenic differences are present then there is a development of more specific T cells that are MHC dependent. These cells mediate the selective, MHC specific cytotoxic response. Despite the development of these cells there are still some of the residual NK-like cells present and these are capable of cross reacting with the broad, common determinants present on the cells within the species (Mawas et al., 1973; Kristensen, 1978).

BIBLIOGRAPHY

Abbasi, K., Demant, P., Festenstein, H., Holmes, J., Huber, B. and Rychlikova, M. 1973. Mouse mixed lymphocytes reactions and cell-mediated lympholysis: Genetic control and relevance to antigenic strength. Transplant. Proc. 5:1329-1337.

Albrechtsen. D., Arhesen, E. and Thorsby, E. 1979. Cell mediated lymphocytotoxicity directed against HLA-D gene products. Transplantation 27:338-341.

Alter, B.J., Schendel, D.J., Bach, M.L., Bach, F.H., Klein, J. and Stimpfling, J.H. 1973. Cell mediated lympholysis-Importance of serologically defined H-2 regions. J. Exp. Med. 137:1303-1309.

Amos, D.B. and Bach, F.H. 1968. Phenotypic expressions of the major histocompatibility locus in man (HL-A): Leukocyte antigens and mixed leukocyte culture reactivity. J. Exp. Med. 128:623-637.

Bach, F.H. 1973. The major histocompatibility complex in transplantation immunology. Transplant. Proc. 5:23-29.

Bach, F.H. and Hirschorn, K. 1964. Lymphocyte interaction: A potential histocompatibility test in vitro. Science 143:813-814.

Bach, F.H. and Segall, M. 1972. Genetics of the mixed leukocyte culture response: A reexamination. Transplant. Proc. 4:205-208.

Bach, F.H., Widmer, M.B., Bach, M.L. and Klein, J. 1972a. Serologically defined and lymphocyte-defined components of the major histocompatibility complex in the mouse. J. Exp. Med. 136:1430-1444.

Bach, F.H., Widmer, M.B., Segall, M. , Bach, M.L. and Klein, J. 1972b. Genetic and immunological complexity of major histocompatibility regions. Science 176:1024-1027.

Bach, F.H., Bach, M.L. and Sondel, P.M. 1976. Different function of major histocompatibility complex antigens in T-lymphocyte activation. Nature 259:273-281.

Bach, F.H., Kuperman, O.J., Sollinger, H.W., Zarling, J.M., Sondel, P.M., Alter, B.J. and Bach, M.L. 1977. Cellular immunogenetics and LD-CD collaboration. Transplant. Proc. 9:859-863.

Baldwin, W.M., Class, F.H.J., vanEs, L.A. and vanRood, J.J. 1980. HLA-A, B, DR and endothelial-specific antigens in kidney-graft rejection. Immunol. Today 1:110-111.

Ball, E.J. and Stastny, P. 1982. Cell mediated cytotoxicity against the HLA-D region products expressed in monocytes and B lymphocytes. IV. Characterization of effector cells using monoclonal antibodies against human T cell subsets. Immunogenetics 16:157-169.

Benacerraf, B. 1978. A hypothesis to relate the specificity of T lymphocytes and the activity of I region-specific Ir genes in macrophages and B lymphocytes. J. Immunol. 120:1809-1812.

Berke, G. and Amos, D.B. 1973. Mechanisms of lymphocyte mediated cytolysis. The LMC cycle and its role in transplantation immunity. Transplant. Rev. 17:71-107.

Berke, G. and Gabison, D. 1975. Energy requirements of the binding and lytic steps of T lymphocyte mediated cytolysis of leukemic cells in vitro. Eur. J. Immunol. 5:671-675.

Berke, G., Sullivan, K.A. and Amos, D.B. 1972. Rejection of ascites tumor allografts. II. A pathway for cell-mediated tumor destruction in vitro by peritoneal exudate lymphoid cells. J. Exp. Med. 136:1594-1604.

Bevan, M.J. 1975. Interaction antigens detected by cytotoxic T cells with the major histocompatibility complex as modifier. Nature 256:419-421.

Bevan, M. 1977. Killer cells reactive to altered self antigens can also be alloreactive. Proc. Natl. Acad. Sci. USA. 74:2094-2098.

Beverley, P.C.L. and Callard, R.E. 1981. Distinctive functional characteristics of human 'T' lymphocytes defined by E-rosetting or a monoclonal anti-T cell antibody. Eur. J. Immunol. 11:329-334.

Binz, H. and Wigzell, H. 1975. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants. I. Demonstration of similar or identical idiotypes on IgG molecules and T cell receptors with specificity for the same alloantigens. J. Exp. Med. 142:197-211.

Binz, H., Lindemann, J. and Wigzell, H. 1974. Cell bound receptors for alloantigens on normal lymphocytes. II. Antialloantibody serum contains specific factors reacting with relevant immunocompetant T lymphocytes. J. Exp. Med. 140:731-741.

Binz, H., Frischknecht, H., Mercolli, C., Dunst, S. and Wigzell, H. 1979. Binding of purified, soluble major histocompatibility complex polypeptide chains onto isolated T-cells receptors. I. Reactivity against allo- and self-reactive determinants. J. Exp. Med. 150:1084-1097.

Boyse, E.A., Miyazawa, M., Aoki, T. and Old, L.J. 1968. Ly-A and Ly-B: Two systems of lymphocyte isoantigens in the mouse. Proc. R. Soc. London, Ser. B. 170:175-193.

Boyse, E.A., Itakura, K., Stockert, E., Iritani, C.A. and Muira, M. 1971. Ly-C: A third locus specifying alloantigens expressed only on thymocytes and lymphocytes. Transplantation 11:351-353.

Boyum, A. 1969. Separation of leucocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. (suppl.) 21:1-108.

Brunner, K.T., Mauel, J., Cerottini, J.C. and Chapuis, B. 1968. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogeneic target cells in vitro; Inhibition by isoantibody and by drugs. Immunology 14:181-196.

Brunner, K.T., Mauel, J., Rudolf, H. and Chapuis, B. 1970. Studies of allograft immunity in mice. I. Induction, development and in vitro assay of cellular immunity. Immunology 18:501-515.

Burakoff, S.J., Germain, R.N. and Benacerraf, B. 1976. Cross-reactive lysis of trinitrophenyl(TNP)-derivatized H-2 incompatible target cells by cytolytic T lymphocytes generated against syngeneic TNP spleen cells. J. Exp. Med. 144:1609-1620.

Burnet, F.M. 1970. The concept of immunological surveillance. Progr. Exp. Tumor Res. 13:1-27.

Burton, R.C., Chism, S.E. and Warner, N.L. 1977. {In} {vitro} induction of tumor-specific immunity. III. Lack of a requirement for H-2 compatibility in lysis of tumor targets by T cells activated in vitro to oncofetal and plasmacytoma antigens. J. Immunol. 118:971-980.

Callard, R.E. and Beverley, P.C.L. 1981. IgG binding T cells. Immunol. Today 2:ii.

Cantor, H. and Asofsky, R. 1970. Synergy among lymphoid cells mediating the graft-versus-host response. II. Synergy in graft-versus-host reactions produce by BALB/C lymphoid cells of differing anatomic origin. J. Exp. Med. 131:235-246.

Cantor, H. and Jandinski, J. 1974. The relationship of cell division to the generation of cytotoxic activity in mixed lymphocyte culture. J. Exp. Med. 140:1712-1716.

Cantor, H. and Boyse, E.A. 1975a. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T cell subclasses is a differentiative process independent of antigen. J. Exp. Med. 141:1376-1389.

Cantor, H. and Boyse, E.A. 1975b. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly^+ cells in the generation of killer activity. J. Exp. Med. 141:1390-1399.

Cantor, H. and Gershon, R.K. 1979. Immunological circuits: Cellular composition. Fed. Proc. 38:2058-2064.

Ceppellini, R., Mattuiz, P.L., Scupeller, G. and Visetti, M. 1969. Experimental allotransplantation in man. I. The role of the HL-A system in different genetic combinations. Transplant. Proc. 1:385-389.

Cerottini, J.C. and Brunner, K.T. 1974. Cell mediated cytotoxicity, allograft rejection and tumor immunity. Adv. Immunol. 18:67-132.

Cerottini, J.C., Nordin, A.A. and Brunner, K.T. 1970. Specific in vitro cytotoxicity of thymus derived lymphocytes sensitized to alloantigens. Nature 228:1308-1309.

Cerottini, J.C., Engers, H.D., MacDonald, H.R. and Brunner, K.T. 1974. Generation of cytotoxic T lymphocytes in vitro. I. Response of normal and immune mouse spleen cells in mixed leukocyte culture. J. Exp. Med. 140:703-717.

Chan, P.L. and Sinclair, N.R. StC. 1971. Regulation of the immune response. V. An analysis of the function of the Fc portion of antibody with respect to interaction with components of the lymphoid system. Immunology 21:967-981.

Chan, P.L. and Sinclair, N.R. StC. 1973. Regulation of the immune response. VI. Inability of $F(ab')_2$ antibody to terminate established immune responses and its ability to interfere with IgG antibody mediated immunosuppression. Immunology 24:289-301.

Chapuis, B. and Brunner, K.T. 1971. Cell mediated immune reactions 'In Vitro'-Reactivity of lymphocytes from animals sensitized to chicken erythrocytes, tuberculin, or transplantation antigens. Inst. Arch. Allergy Appl. Immunol. 40:321-339.

Claman, H.N. and Chaperon, E.A. 1969. Immunologic complementation between thymus and marrow cells- A model for the two-cell theory of immunocompetence. Transplant. Rev. 1:92-113.

Clark, P.A., Phillips, R.A. and Miller, R.G. 1976. Characterization of cells that suppress the cytotoxic activity of T lymphocytes. I. Quantitative measurement of inhibitor cells. J. Immunol. 116:1020-1029.

Cochrum, K.C., Perkins, H.A., Payne, R.O., Kountz, S.L . and Beltzer, F.O. 1973. The correlation of MLC with graft survival. Transplant. Proc. 5:391-396.

Cohen, L. and Howe, M.L. 1973. Synergism between subpopulations of thymus-derived cells mediating the proliferative and effector phases of the mixed lymphocytes reaction. Proc. Natl. Acad. Sci. USA. 70:2707-2710.

Collins, J.K., Britt, W.J. and Chesbro, B. 1980. Cytotoxic T lymphocytes recognition of gp70 on Friend virus-induced erythro-leukemia cell clones. J. Immunol. 125:1318-1323.

Cooper, E.L. 1965. A method of tissue grafting in the earthworm Lumbricus Terrestris. Amer. Zool. 5:254.

Cooper, M.D., Peterson, R.D.A. and Good, R.A. 1965. Delineation of the thymic and bursal lymphoid systems in the chicken. Nature 205:143-146.

Dausset, J. 1958. Iso-leuco-anticorps. Acta. Haemat. (Basal) 20:156-166.

Davies, A.J.S. 1969. The thymus and the cellular basis of immunity. Transplant. Rev. 1:43-91.

Davis, B.D., Dulbecco, R., Eisen, H.N., Ginsberg, H.S. and Wood, B.W. 1973. In 'Microbiology' pg. 570, second edition, Harper and Row.

Devens, B., Schochot, L. and Noar, D. 1979. Immune responses to weakly immunogeneic virally induced tumors. V. Short in vitro cultivation of YAC changes its antigenic properties. Cell. Immunol. 44:442-453.

Dickmeiss, E., Soeberg, B. and Svejgaard, A. 1977. Human cell-mediated cytotoxicity against modified target cells is restricted by HLA. Nature 270:526-528.

Doherty, P.C., Effros, R.B. and Bennink, J. 1977. Heterogeneity of the cytotoxic response of thymus-derived lymphocytes after immunization with influenza viruses. Proc. Natl. Acad. Sci. USA. 74:1209-1213.

Duc, H.T., Kinsky, R.G. and Voisin, G.A. 1978. Ia versus K/D antigens in immunological enhancement of tumor allografts. Transplantation 25:182-187.

Duc, H.T., Kinsky, R.G. and Voisin, G.A. 1979. Ia versus K/D antigens in immunological enhancement of tumor allografts. II. Studies with alloimmune sera prepared in recombinant strains. Ann. Immunol. (Inst. Pasteur) 130:461-474.

Edidin, M. and Henney, C.S. 1973. The effect of capping H-2 antigens on the susceptibility of target cells to humoral and T cell mediated lysis. Nature, New Biology. 246:47-49.

Eijsvoogel, V.P., Koning, L., deGroot-Kooy, L., Huismans, L., vanRood, J.J., vanLeeuwen, A. and duToit, E.D. 1972. Mixed lymphocyte culture and HL-A. Transplant. Proc. 4:199-204.

Eijsvoogel, V.P., duBois, R., Meleif, C.J.M., Zeijlemaker, W.P., Raat-Koning, L. and deGroot-Kooy, L. 1973. Lymphocyte activation and destruction in vitro in relation to MLC and HL-A. Transplant. Proc. 5:415-420.

Eijsvoogel, V.P., Schellekens, P.T.A., duBois, M.J. and Zeijlemaker, W.P. 1976. Human cytotoxic lymphocytes after alloimmunization in vitro. Transplant. Rev. 29:125-145.

Evans, R. and Alexander, P. 1972. Mechanism of immunologically specific killing of tumor cells by macrophages. Nature (Lond.) 236:168-170.

Feldmann, M., Cohen, I.R. and Wekerle, H. 1972. T-cell mediated immunity in vitro: An analysis of antigen recognition and target cell lysis. Transplant. Rev. 12:57-90.

Ferluga, J. and Allison, A.C. 1974. Observation on the mechanism by which T-lymphocytes exert cytotoxic effects. Nature 240:673-675.

Festenstein, H. 1974. Pertinant features of M locus determinants including revised nomenclature and strain distribution. Transplantation 18:555-557.

Finberg, R., Burakoff, S.J., Cantor, H. and Benacerraf, B. 1978. Biological significance of alloreactivity: T cells stimulated by Sendai virus-coated syngeneic cells specifically lyse allogeneic target cells. Proc. Natl. Acad. Sci. USA. 75:5145-5149.

Fink, P.J. and Bevan, M.J. 1978. H-2 antigens of the thymus determine lymphocyte specificity. J. Exp. Med. 148:766-775.

Finstad, J. and Good, R.A. 1966. In 'Phylogeny of Immunity', Smith, R.T., Meischler, P.A. and Good, R.A. (eds.) University of Florida Press, Gainsville. pg. 173-188.

Flax, M.H. and Caulfield, J.B. 1963. Cellular and vascular components of allergic contact dermititis. Am. J. Pathol. 43:1031-1054.

Forman, J., Vitetta, E.S., Hart, D.A. and Klein, J. 1977a. Relationship between trinitrophenyl and H-2 antigens on trinitrophenyl-modified spleen cells. I. H-2 antigens on cells treated with trinitrobenzene sulfonic acid are derivatized. J. Immunol. 118:797-802.

Forman, J., Vitetta, E.S. and Hart, D.A. 1977b. Relationship between trinitrophenyl and H-2 antigens on trinitrophenyl-modified spleen cells. II. Correlation between derivitization of H-2 antigens with trinitrophenyl and the ability of trinitrophenyl-modified cells to react functionally in the CML assay. J. Immunol. 118:803-808.

Fradelizi, D. and Dausset, J. 1975. Mixed lymphocyte reactivity of human lymphocytes primed in vitro. I. Secondary response to allogeneic lymphocytes. Eur. J. Immunol. 5:295-301.

Freidman, S.M., Heyhard, N. and Chess, L. 1978. Cell mediated lympholysis of trinitrophenyl derivatized autologous human cells: In vitro triggering by non-specific signals. J. Immunol. 120:630-637.

Freidman, S.M., Kuhns, J., Irigoyen, O. and Chess, L. 1979. The induction of TNP-altered, self-reactive human cytotoxic T cells by soluble factors: The role of Ia antigens. J. Immunol. 122:1302-1309.

Fudenberg H.H., Pink, J.R.L., Wong, A.C. and Douglas, S.D. 1978. Basic Immunogenetics. Oxford University Press, New York, N.Y.

Fujimoto, S., Greene, M.I. and Sehon, A.H. 1976. Regulation ot the immune response to tumor antigens. II. Immunosuppressor cells in tumor-bearing hosts. J. Immunol. 116:791-799.

Gatti, R.A., Stutman, O. and Good, R.A. 1970. The lymphoid system. Ann. Rev. Physiol. 32:529-546.

Geha, R.S., Malakian, A., Geha, O. and Yunis, E.J. 1977. Genetics of CML in man. J. Immunol. 118:1286-1291.

Gluckman, J.C., Gluckman, E., Andersen, E. and Rottenbourg, J. 1977. Cellular presensitization to alloantigens in haemodialyzed patients. Transplantation 23:65-72.

Golstein, P., Svedmyr, E.A.J. and Wigzell, H. 1971. Cells mediating specific in vitro cytotoxicity. I. Detection of receptor bearing lymphocytes. J. Exp. Med. 134:1385-1402.

Golstein, P., Wigzell, H., Blomgren, H. and Svedmyr, E.A.J. 1972. Cells mediating specific in vitro cytotoxicity. II. Probable autonomy of thymus-processed lymphocytes (T-cells) for killing of allogeneic target cells. J. Exp. Med. 135:890-906.

Golstein, P., Lucian, M. and Rubin, B. 1980. Xenogeneic serum-induced mouse T cells that trigger the differentiation of precursor into cytolytic T cells. J. Immunol. 125:1144-1151.

Good, R.A. Finstad, J., Pollara, B. and Gabrielsen, A.E. 1966. In 'Phylogeny of Immunity', Smith, R.T., Mieschler, P.A. and Good, R.A. (eds.) University of Florida Press, Gainesville. Pg. 159-168.

Gorer, P.A. 1937. The genetic and antigenic basis of tumor transplantation. J. Path. Bact. 44:591-697.

Gorer, P.A. and Mikulka, Z.B. 1954. The antibody response to tumor inoculation. Improved methods of antibody detection. Cancer Res. 14:651-655.

Goulmy, E., Termijtelan, A., Bradley, B.A. and vanRood, J.J. 1977. Y-antigen killing by T cells of woman is restricted by HLA. Nature 266:544-545.

Govaerts, A. 1960. Cellular antibodies in kidney homotransplantation. J. Immunol. 85:516-522.

Granger, G.A. and Kolb, W.D. 1968. Lymphocyte in vitro cytotoxicity: Mechanisms of immune and non-immune small lymphocyte mediated target L cells destruction. J. Immunol. 101:111-120.

Greenberg, A.H. and Greene, M. 1976. Non-adaptive rejection of small tumor inocula as a model of immune surveillance. Nature (Lond.) 264:356-359.

Greenberg, J.M. and Takasugi, M. 1980. Human natural cell mediated cytotoxicity: A polyspecific system. In 'Natural Cell Mediated Immunity Against Tumors', R.B. Herberman (ed.), Academic Press, New York, N.Y. pg.835-853.

Grunnet, N., Kristensen, T., Konerup, H.J. and Kissmeyer-Neilsen, F. 1975. Direct cell mediated lympholysis. A test of allograft rejection in human kidney recipients. Tissue Antigens 5:280-285.

Grunnet, N., Kristensen, T. and Kissmeyer-Neilsen, F. 1976. Cell mediated lympholysis in man: The impact of HLA-C antigens. Tissue Antigens 7:301-307.

Halgrimson, C.G., Rapaport, F.T., Terasaki, P.I., Porter, K.A., Andres, G., Penn, I., Putnam, C.W. and Starzl, T.E. 1971. Net histocompatibility ratios (NHR) for clinical transplantation. Transplant. Proc. 3:140-144.

Hammerling, G.J. and McDevitt, H.O. 1974. Antigen binding T and B lymphocytes. II. Studies on the inhibition of antigen binding to T and B cells by anti-immunoglobulin and anti-H-2 sera. J. Immunol. 112:1734-1740.

Haynes, B.F., Mann, D.L., Hemler, M.E., Schroer, H.A., Shelhamer, H.H., Eisenbarth, G.S., Strominger, J.L., Thomas, C.A., Mostowski, H.S. and Fauci, A.S. 1980. Characterization of a monoclonal antibody that defines an immunoregulatory T cell subset for immunoglobulin synthesis in humans. Proc. Natl. Acad. Sci. USA. 77:2914-2916.

Hayry P. and Defendi, V, 1970. Mixed lymphocyte cultures produce effector cells: An in vitro model for allograft rejection. Science 168:133-136.

Hayry, P. and Andersson, L.C. 1974. T cell synergy in mixed lymphocyte culture-induced cytolysis. Eur. J. Immunol. 4:145-147.

Henney, C.S. 1973. On the mechanism of T cell mediated cytolysis. Transplant. Rev. 17:37-70.

Henney, C.S., Clayburg, J., Cole, G.A. and Prendergast, R.A. 1972. B-lymphocyte mediated cytolysis: A complement independent phenomenon. Immunol. Comm. 1:93-103.

Henney, C.S., Kuribayashi, K., Kerm, D.E. and Gillis, S. 1981. Interleukin-2 augments natural killer cell activity. Nature (Lond.) 291:335-338.

Hodes, R.J. and Svedmyr, E.A.J. 1970. Specific cytotoxicity of H-2 incompatible mouse lymphocytes following mixed culture in vitro. Transplantation 9:470-477.

Hodes, R.J. and Hathcock, K.S. 1976. In vitro generation of suppressor cell activity: Suppression of in vitro induction of cell mediated cytotoxicity. J. Immunol. 116:167-177.

Hoffmann, M.K., Kappler, J.W., Hirst, J.A. and Oettgen, H.F. 1974. Regulation of the immune response. V. Antibody mediated inhibition of T and B cell cooperation in the in vitro response to red cell antigens. Eur. J. Immunol. 4:282-286.

Hoffmann, R.A., Kung, P.C., Hansen, W.P. and Goldstein, G. 1980. Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc. Natl. Acad. Sci. USA. 77:4914-4917.

Holm, G. and Perlmann, P. 1967. Quantitative studies on phytohaemagglutinin-induced cytotoxicity by human lymphocytes against homologous cells in tissue culture. Immunology 12:525-536.

Holter, A.R., Neu, M.R., McKearn, T.J., Lynch, A.F and Stuart, F.P. 1973. Abrogation of hyperacute rejection of renal allografts by pepsin digest fragments of anti-donor antibody. Transplant. Proc. 5:593-596.

Howe, M.L. and Ghea, L. 1975a. Lymphoid cell subpopulations. I. Synergy between lymph node cells and thymocytes in response to alloantigens and mitogens. J. Immunol. 115:1227-1232.

Howe, M.L. and Ghea, L. 1975b. Lymphoid cell subpopulations. II. Characterization of cell populations responsible for synergy in the mixed lymphocyte interaction. J.Immunol. 115:1233-1238.

Janeway, C.A. Jr. 1976. The specificity of T lymphocyte responses to chemically defined antigens Transplant. Rev. 29:164-188.

Jansen, J.L.J., Koene, R.A.P., Kamp, G., Gerlag, P.G.G. and Wijdeveld, P.G.A.B. 1975. Enhancement and hyperacute rejection of skin grafts in the mouse. Failure to separate cytotoxic and enhancing antibodies from alloantiserum with physiochemical methods. J.Immunol. 114:1557-1562.

Jondal, M., Holm, G. and Wigzell, H. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. J. Exp. Med. 136:207-215.

Jones, B. and Jones, T.C. 1978. Allograft cytotoxicity cooperation between alloimmune T cells and macrophages. Immunology 35:247-255.

Kaldany, A., Carpenter, C.B., Shadur, C.A., George, K., Lundin, A.P., Suthanthiran, M. and Strom, T.B. 1980. Immunological properties of subcellular rat lymphocyte preparations: Primary allogeneic stimulation in vitro by fractions containing Ia(RTI-B), but not RTI-A antigens. J. Exp. Med. 151:910-924.

Kaplan, J. and Calleweart, D.M. 1978. Expression of human T lymphocyte antigens by natural killer cells. J. Natl. Cancer Inst. 60:961-964.

Kaplan, J. and Calleweart, D.M. 1980. Are natural killer cells germline V-gene encoded prothymocytes specific for self and non-self histocompatibility antigens. In 'Natural Cell Mediated Immunity Against Tumors', R.B. Herberman (ed.), Academic Press, New York, N.Y. pg:893-908.

Kay, H.D., Bonnard, G.D. and Herberman, R.B. 1979. Evaluation of the role of anti-IgG antibodies in human natural cell mediated cytotoxicity against the myeloid cell line K562. J. Immunol. 1979. 122:675-685

Kennedy, L.J. Jr., Dorf, M.E., Unanue, E.R. and Benacerraf, B. 1975. Binding of poly($Glu^{60}Ala^{30}Tyr^{10}$) by thymic lymphocytes from genetic responder and non-responder mice: Effect of anti-histocompatibility serum. J. Immunol. 114:1670-1675.

Klein, E. 1981. Central issues in present day tumor immunology; Interpretation of lymphocytotoxicity assays and demonstration of auto-tumor reactive lymphocytes in patients. Transplant. Proc. 13:723-728.

Klein, G., Sjorgen, H.O., Klein, E. and Hellstrom, K.E. 1960. Demonstration fo resistance against methylcholanthrene-induced sarcomas in the primary autochthontous host. Cancer Res. 20:1561-1572.

Klein, J. and Schreffler, D.C. 1971. The H-2 model for the major histocompatibility systems. Transplant. Rev. 6:3-29.

Kuichi, M. and Takasugi, M. 1976. The non-selective cytotoxic cell (N cell). J. Natl. Cancer Inst. 56:575-582.

Komoro, K., Itakura, K., Boyse, E.A. and John, M. 1975. Ly-5: A new T-lymphocyte antigen system. Immunogenetics 1:452-456.

Kristensen, T., Grunnet, N. and Kissmeyer-Nielsen, F. 1974a. Cell mediated lympholysis in man. Occurrence of unexpected, HL-A (LA and FOUR) irrelevant lympholysis. Tissue Antigens 4:378-382.

Kristensen, T., Grunnet, N., Thorsen, Inge-Lis and Kissmeyer-Nielsen, F. 1974b. Cell mediated lympholysis in man. The non-impact of the ABO-bloodgroup system. Tissue Antigens 4:541-547.

Kristensen, T., Grunnet, N. and Kissmeyer-Nielsen, F. 1975. Cell mediated lympholysis in man. Varying strengths of the HLA (LA and FOUR) antigens as sensitizing or target determinants. Tissue Antigens 6:221-228.

Kristensen, T., Grunnet N. and Kissmeyer-Nielsen, F. 1976a. Cell mediated lympholysis in man. Discriminatory sensitizing and effector potential of lymphocytes from three individuals. Transplantation 21:337-340.

Kristensen, T., Grunnet, N., Jorgensen, F., Lamm, L. and Kissmeyer-Nielsen, F. 1974b. Cell mediated lympholysis in man. An attempt to type with cytotoxic lymphocytes. Tissue Antigens 8:299-316.

Kristensen, T. 1978. Studies on the specificity of CML: Report from a CML-workshop. Tissue Antigens. 11:330-349.

Kuribayashi, K., Gillis, S., Kern, D.E. and Henney, C.S. 1981. Murine NK cell cultures: Effect of interleukin-2 and interferon on cell growth and cytotoxic reactivity. J. Immunol. 126:2321-2327.

Landsteiner, K. and Chase, M.W. 1942. Experiments on transfer of cutaneous sensitivity to simple compounds. Proc. Soc. Exp. Biol. Med. 49:688-690.

Lawrence, H.S. 1957. Similarities between homograft rejection and tuberculin-type allergy: A review of recent experimental findings. Ann. N.Y. Acad. Sci. 64:826-832.

Lemonnier, F., Burakoff, S.J., Mescher, M., Dorf, M.E. and Benacerraf, B. 1978. Inhibition of the induction of cytolytic T lymphocytes with alloantisera directed against H-2K and H-2D gene products. J. Immunol. 120:1717-1720.

Lohmann-Matthes, M., Schipper, H and Fischer, H. 1972. Macrophage-mediated cytotoxicity against allogenic target cells *in vitro*. Eur. J. Immunol. 2:45-49.

Long, M.A., Handwerger, B.S., Amos, D.B. and Yunis, E.J. 1976. The genetics of cell mediated lympholysis. J.Immunol. 117:2092-2099.

Loop, S.M., Bernstein, I.D. and Wright, D.W. 1980. T cell synergy in the rat: Serologic characterization of T cell subsets J. Immunol. 125:1237-1239.

Lung, P.C. and Singal, D.P. 1981. Functional roles of T cell subpopulations in T-T cell interactions. Transplant. Proc. 13:1160-1163.

MacDonald, H.R. 1975. Early detection of potentially lethal events in T cell mediated cytolysis. Eur. J. Immunol. 5:251-254.

Mackaness, G.B. and Blanden, R.V. 1967. Cellular immunity. Prog. Allergy 11:89-140.

Marbrook, J. 1967. Primary immune response in cultures of spleen cells. Lancet 2:1279-1281.

Martz, E. and Benacerraf, B. 1975. T-lymphocyte mediated cytolysis: Temperature dependence of killer cells dependent and independent phases and lack of recovery from the lethal hit at low temperatures. Cellular Immunol. 20:81-91.

Matter, A. 1979. Microcinematographic and electron microscope analysis of target cell lysis induced by cytolytic T lymphocytes. Immunol. 36:179-190.

Mawas, C., Sasportes, M., Cristen, Y., Bernard, A., Dausset, J., Alter, B.J. and Bach, M.L. 1973. Cell-mediated lympholysis (CML) in the absence of LD2 mixed lymphocyte reaction and CML in the presence of SD1-SD2 identity in two HL-A genotyped families. Transplant. Proc. 5:1683-1689.

Mawas, C., Christen, Y., Legrand, L. Sasportes, M. and Dausset, J. 1974. Cellular and humoral responses against determinants other than the classical HL-A specificities. Evidence for at least one system independent of the major histocompatibility complex. Transplantation 18:256-266.

McKearn, T.J. 1974. Antireceptor antiserum causes specific inhibition of reactivity to rat histocompatibility antigens. Science 183:94-96.

McMichael, A.J., Ting, A., Zweerink, H.J. and Askonas, B.A. 1977. HLA restriction of cell mediated lysis of influenza virus infected human cells. Nature 270:524-526.

Meo, T., Vives, J., Miggiano, V. and Schreffler, D. 1973. A major role for the Ir-1 region of the mouse H-2 complex in the mixed leukocyte reaction. Transplant. Proc. 5:377-381.

Miggiano, V.C., Bernoco, D., Lightbody, J., Trinchieri, G. and Ceppellini, R. 1972. Cell-mediated lympholysis in vitro with normal lymphocytes as target: Specificity and cross-reactivity of the test. Transplant. Proc. 4:231-237.

Miller, J.F.A.P. and Mitchell, G.F. 1969. Thymus and antigen reactive cells. Transplant. Rev. 1:3-42.

Moller, E. 1965. Antagonistic effects of humoral isoantibodies on the in vitro cytotoxicity of immune lymphoid cells. J. Exp. Med. 122:11-23.

Mond, J.J., Kaplan, R.E. and Thorbecke. G.J. 1973. Effect in vitro of anti-immunoglobulin sera on proliferative responses of lymphoid cells to antigen. Eur. J. Immunol. 3:153-156.

Moretta, L. and Fauci, A.S. 1980. Subpopulations of human T lymphocytes. Immunol. Today 1:3-4.

Moretta, L., Ferrarini, M., Mingari, M.C., Moretta, A. and Webb, S.R. 1976. Subpopulations of human T cells identified by receptors for immunoglobulins and mitogen responsiveness. J.Immunol. 117:2171-2174.

Moretta, L., Moretta, A., Canonica, G.W., Bacigalupo, A., Mingari, M.C. and Cerotinni, J.C. 1981. Receptors for immunoglobulins on resting and activated human T cells. Immunological Rev. 56:141-162.

Nagy, Z., Elliot, B.E., Habholz, M., Krammer, P.H. and Pernis, B. 1976a. Specific binding of alloantigens to T cells activated in the mixed lymphocyte reaction. J. Exp. Med. 143:648-659.

Nagy, Z., Elliot, B.E. and Nabholz, M. 1976b. Specific binding of K and I-region products of the H-2 complex to activated thymus-derived (T) cells belonging to different Ly subclasses. J. Exp. Med. 144:1545-1553.

Netzel, B., Grosse-Wilde, H., Baumann, P. and Mempel, W. 1975. LD typing in man using cells frozen and stored in microtiter plates. Tissue Antigens 6:8-14.

Neveu, D.J., Micusan, V.V., Farfard, A. and Borduas, A.G. 1978. Modulation of the immune response by passive antibodies. IV. Effects of IgG1 and IgG2 anti-hapten antibodies. Immunology 35:129-132.

Nielsen, H.E., Heron, I. and Koch, C. 1977. Inhibition of the mixed leucocyte reaction by histocompatibility antibodies requires the Fc part. Transplantation 23:73-77.

Oberbarnscheidt, J. and Kolsch, E. 1978. Direct blockade of antigen reactive B lymphocytes by immune complexes. An 'off' signal for precursors of IgM producing cells provided by the linkage of antigen and Fc receptors. Immunology 35:151-157.

Ortaldo, J. and Herberman, R.B. 1980. In vitro development of human NK cells: Characteristics of precursors and effector cells and possible cell lineage. pg.465-476. In 'Natural Cell Mediated Immunity Against Tumors', R.B. Herberman (ed.), Academic Press Inc., New York, N.Y. pg.465-476.

Paul, W.E., Shevach, E.M., Thomas, D.W., Pickerac, S.F. and Rosenthal, A.S. 1976. Genetic restriction in T lymphocyte activation by antigen pulsed peritoneal exudate cells. Cold Spring Harbor Symp. Quant. Biol. 41:571-578.

Peck, A.B., Klein, J. and Wigzell, H. 1980. The mouse-primed lymphocyte typing test. III. Dissociation of T lymphocyte stimulating determinanats and antibody defined specificities of the I region associated Ia determinants. J. Immunol. 125:1078-1086.

Penfold, P.L. and Jones, B. 1979. Allograft cytotoxicity: Differences between lytic and non-lytic interactions as revealed by ultrastructural histochemistry. Immunology 36:509-518.

Perlmann, P., Perlmann, H. and Wigzell, H. 1972. Lymphocyte mediated cytotoxicity in vitro. Induction and inhibition by humoral antibody and nature of effector cells. Transplant. Rev. 13:91-114.

Pichler, W.J. and Broder, S. 1981. In vitro functions of human T cells expressing Fc-IgG or Fc-IgM receptors. Immunological Rev. 56:163-197.

Plate J.M.D. and McKenzie, I.F.C. 1973. 'B'-cell stimulation of allogeneic T cell proliferation in mixed lymphocyte cultures. Nature (Lond.) New Biol. 245:247-249.

Plaut, M., Lichenstein, L.M., Gillespie, E. and Henney, C.S. 1973. Studies on the mechanisms of lymphocyte. III. The role of microfilaments and microtubules. J. Immunol. 110:771-780.

Pross, H.F. and Baines, M.G. 1980. Characteristics of human natural killer cells. In 'Natural Cell Mediated Immunity Against Tumors'. R.B. Herberman (ed.), Academic Press Inc., New York, N.Y. pg.151-159.

Ramseier, H. and Lindemann J. 1972. Allotypic antibodies. Transplant. Rev. 10:57-96.

Reinherz, E.L., Moretta, L., Roper, M., Breard, J.M., Minagari, M.C., Cooper, M.D. and Schlossman, S.F. 1980. Human T lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. J. Exp. Med. 151:969-974.

Roder, J.C. and Haliotis, T. 1980. Do NK cells play a role in anti-tumor surveillance? Immunol. Today 1:96-100.

Rosenau, W. and Moon, H.D. 1961. Lysis of homologous cells by sensitized lymphocytes in tissue culture. J. Natl. Cancer Inst. 27:471-483.

Rubinstein, P., Decary, F. and Steun, E.W. 1974. Quantitative studies on tumor enhancement in mice. I. Enhancement of Sarcoma 1 induced by IgM, IgG1, and IgG2. J. Exp. Med. 140:591-596.

Ruddle, N.H. and Waksman, B.H. 1967. Cytotoxic effect of lymphocyte-antigen interaction in delayed hypersensitivity. Science 157:1060-1061.

Russell J.H. and Dobos, C.B. 1980. Mechanisms of immune lysis. II. CTL induced nuclear disintegration of the target begins within minutes of cell contact. J. Immunol. 125:1256-1261.

Sanderson, C.J. and Glauert, A.M. 1979. The mechanism of T cell mediated cytotoxicity. Immunology 36:119-129.

Sasportes, M., Lebrun, A., Rapaport, F,T. and Dausset, J. 1972. Studies of skin allograft survival and mixed lymphocyte culture reaction in HLA genotyped families. Transplant. Proc. 4:209-218.

Schendel, D.J and Bach, F.H. 1974. Genetic control of cell mediated lympholysis in the mouse. J. Exp. Med. 140:1534-1546.

Schendel, D.J. and Bach, F.H. 1975. H-2 and non-H-2 determinants in the genetic control of cell mediated lympholysis. Eur. J. Immunol. 5:880-882.

Schlossman, S. 1980. Differentiation and function of human T lymphocytes. In 'Immunoregulation and Autoimmunity' R.S. Krakauer and M.K. Cathcart (eds.), Elsevier North Holland, Inc. pg.51-53.

Schmitt-Verhulst, A.M., Sachs, D.H. and Shearer, G.M. 1976. Cell-mediated lympholysis of trinitrophenyl-modified autologous lymphcytes. Confirmation of genetic control of response to trinitrophenyl-modified H-2 antigens by the use of anti-H-2 and anti-Ia antibodies. J. Exp. Med. 143:221-217.

Segall, M. and Bach, F.H. 1976. Pooled stimulating cells as a 'standard stimulator' in mixed lymphocyte culture. Transplantation 22:79-85.

Seksela, E. and Timonen, T. 1980. Morphology and surface properties of human NK cells. In 'Natural Cell Mediated Immunity Against Tumors', R.B. Herberman (ed.). Academic Press Inc., New York, N.Y. pg.173-185.

Shaw, S., Nelson, D.L. and Shearer, G.M. 1978. Human cytotoxic response in vitro to trinitrophenyl-modified autologous cells. I. T cell recognition of TNP in association with widely shared antigens. J. Immunol. 121:281-289.

Shaw, S. and Shearer, G.M. 1978. Human cytotoxic response in vitro to trinitrophenyl-modified autologous cells. II. Diversity of self determinants recognized in association with TNP. J. Immunol. 121:290-299.

Shearer, G.M. 1974. Cell mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. Eur. J. Immunol. 4:527-533.

Shearer, G.M. and Schmitt-Verhulst, A.M. 1977. Major histocompatibility complex restricted cell mediated immunity. Adv. Immunol. 25:55-91.

Shearer, G.M., Rehn, T.G. and Garbarino, C.A. 1975. Cell mediated lympholysis of trinitrophenyl-modified autologous lymphocytes. Effector cell specificity to modified cell surface components controlled by H-2K and H-2D serological regions of the murine major histocompatibility complex. J. Exp. Med. 141:1348-1364.

Shearer, G.M., Rehn, T.G. and Schmitt-Verhulst, A.M. 1976a. Role of the murine major histocompatibility complex in the specificity of in vitro T cell mediated lympholysis against chemically modified autologous lymphocytes. Transplant. Proc. 29:222-248.

Shearer, G.M., Schmitt-Verhulst, A.M. and Rehn, T.G. 1976b. In 'The Role of Products of the Histocompatibility Gene Complex in Immune Responses.', Katz, D.H. and Benacerraf, B. (eds.), Academic Press, New York, N.Y. pg 133.

Sheehy, M.J., Sondel, P.M., Bach, M.L., Wank, R. and Bach, F.H. 1975. HL-A LD (Lymphocyte Defined) typing: A rapid assay with primed lymphocytes. Science 188:1308-1310.

Shustik, C., Cohen, I.R., Schwartz, R.S. and Latham-Griffin, E. 1976. T lymphocytes with promiscuous cytotoxicity. Nature 263:699-701.

Sinclair, N.R.StC. 1969. Regulation of the immune response. I. Reduction of the ability of specific antibody to inhibit long lasting IgG immunological priming after removal of the Fc fragment. J. Exp. Med. 129:1183-1201.

Sinclair, N.R.StC. 1978. Immunoregulation by antibody and antigen-antibody complexes. Transplant. Proc. 10:349-353.

Sinclair, N.R.StC. 1979. Modulation of immunity by antibody, antigen-antibody complexes and antigen. Pharmac. Ther. 4:355.

Sinclair, N.R.StC. and Chan, P.L. 1971. Regulation of the immune response. IV. The role of the Fc fragment in feedback inhibition by antibody. Adv. Exp. Med. Biol. 12:609-615.

Sinclair, N.R.StC., Lees, R.K., Fagan, G. and Birnbaum, A. 1975. Regulation of the immune response. VIII. Characteristics of antibody mediated suppression of an in vitro cell mediated immune response. Cell. Immunol. 16:330-347.

Sinclair, N.R.StC., Lees, R.K., Wheeler, M.E., Vichos, E.E. and Fung, F.Y. 1976a. Regulation of the immune response. XI. Cell mediated feedback of an in vitro cell mediated immune response. Cell. Immunol. 27:153-162.

Sinclair, N.R.StC., Lees, R.K. and Chan, P.L. 1976b. Interference with antibody feedback by irradiation, thymus cells, the allogeneic effect, and serum factors. Exp. Med. Biol. 66:623-633.

Sinclair, N.R.StC., and Law, F.Y. 1979. Antibody mediated immunosuppression of a cytotoxic cell response not involving a simple antigen masking mechanism. J. Immunol. 123:1439-1444.

Singal, D.P., Younglai, E.V. and Naidaul, N. 1975. Inhibition of the mixed leucocyte culture reaction by plasma from renal transplant recipients. Tissue Antigens 5:19-25.

Singal, D.P., Blajchman, M.A., Naipaul, N. and Joseph, S. 1981. Non-HLA antigens in the primed lymphocyte test (PLT): Response to Lewis determinants. Transplant. Proc. 13:1193-1196.

Snell, G.D. 1948. Methods for the study of histocompatibility genes. J. Genet. 49:87-108.

Snell, G.D., Smith, P. and Gabrielson, F. 1953. Analysis of the histocompatibility-2 locus in the mouse. J. Nat. Cancer Inst. 14:457-480.

Solliday, S. and Bach F.H. 1970. Cytotoxicity: Specificity after in vitro sensitization. Science 170:1406-1409.

Sollinger, H.W. and Bach, F.H. 1976. Collaboration between in vivo responses to LD and SD antigens of major histocompatibility complex. Nature 259:487-488.

Sondel, P.M. and Bach, F.H. 1976. Recognitive specificity of human T lymphocytes. II. The non-recognition of antigens controlled outside the major histocompatibility complex. Tissue Antigens 7:173-180.

Sondel, P.M., Chess, L., MacDermott, R.P. and Schlossman, S.F. 1975. Immunologic function of isolated human lymphocyte subpopulations. III. Specific allogeneic lympholysis mediated by human T cells alone. J. Immunol. 114:982-987.

Sondel, P.M., Sparks, E.M., Glass, N.R., Sollinger, H.W. and Belzer, F.O. 1982. Prospective renal allograft matching by pool primed lymphocyte typing. No correlation because of poor responses of prospective recipients. Transplantation 33:224-227.

Staines, N.A., Guy, K. and Davies, D.A.L. 1975. The dominant role of Ia antibodies in the passive enhancement of H-2 incompatible skin grafts. Eur. J. Immunol. 5:782-789.

Stiller, C.R., Sinclair, N.R.StC., Abrahams, S., McGirr, D., Singh, H., Howson, W.T. and Ulan, R.A. 1976. Antidonor immune responses in prediction of transplant rejection. New Eng. J. Med. 294:978-982.

Stuck, B., Boyse, E.A., Old, L.J. and Carswell, E.A. 1964. ML: A new antigen found in leukemias and mammary tumours of the mouse. Nature 203:1033-1034.

Stulting, R.D. and Berke, G. 1973. Nature of lymphocyte-tumor interaction. A general method for cellular immunoabsorption. J. Exp. Med. 137:932-942.

Stutman, O. 1981. NK cells, antitumor surveillance, and interleukins. Immunol. Today 2:205-208.

Stutman, O. and Good, R.A. 1969. Traffic of hemopoietic cells to the thymus: Influences of histocompatibility differences. Exp. Hematol. 19:12-15.

Suciu-Foca, N. and Dausset, J. 1975. Mixed lymphocyte cultures in a family with an LD allele shared by the parents. Tissue Antigens 5:137-141.

Sy, M.S., Brown, A.R., Benacerraf, B. and Greene, M.I. 1980. Antigen and receptor driven regulatory mechanisms. III. Induction of delayed type hypersensitivity to azobenzene-arsonate with anti-cross-reactive idiotypic antibodies. J. Exp. Med. 151:896-909.

Takasugi, M. and Mickey, M.R. 1976. Interaction analysis of selective and nonselective cell mediated cytotoxicity. J. Natl. Cancer Inst. 57:255-261.

Terasaki, P.I., ed. 1970. 'Histocompatibility Testing-1970', Munksgaard, Copenhagen.

Third Conference on Human Gene Mapping, Baltimore, 1975.

Ting, A. 1981. The effect of HLA matching on kidney graft survival. Immunol. Today 2:25-29.

Trinchieri, G., Bernocco, D., Curtoni, S.E., Miggiano, V.C. and Ceppellini, R. 1972. Cell mediated lympholysis in man: Relevance of HLA antigens and antibodies. In 'Histocompatibility Testing, 1972', P.I. Terasaki (ed.), Munksgaard, Copenhagen.

Uhr, J.W. and Moller, G. 1968. Regulatory effect of antibody on the immune response. Adv. Immunol. 8:81-127.

vanRood, J.J., vanLeeuwen, A., Keuning, J.J. and Blusse vanOud Albais, A. 1975. The serological recognition of the human MLC determinants using a modified cytotoxicity technique. Tissue Antigens 5:73-79.

vanSomeren, H., Westerveld, A., Hagemeyer, A., Mees, J.R., Meerakhan, P. and Zaalberg, O.B. 1974. Human antigen and enzyme markers in man/chinese hampster somatic cell hybrids. Proc. Natl. Acad. Sci. (Wash.) 71:962-969.

Voisin, G.A. 1980. Role of antibody classes in the regulatory facilitation reaction. Immunol. Rev. 49:3-59.

Wagner, H. 1973. Synergy during in vitro cytotoxic allograft responses. I. Evidence for cell interaction between thymocytes and peripheral T cells. J. Exp. Med. 138:1379-1397.

Wagner, H. and Rollinghoff, M. 1973. In vitro induction of tumor specific immunity. I. Parameters of activation and cytotoxic reactivity of mouse lymphoid cells immunized in vitro against syngeneic and allogeneic plasma cell tumors. J. Exp. Med. 138:1-15.

Wagner, H. and Rollinghoff, M. 1974. T cell mediated cytotoxicity: Discrimination between antigen recognition, lethal hit, and cytolysis phase. Eur. J. Immunol. 4:745-750.

Wagner, H., Harris, A.W. and Feldmann, M. 1972. Cell mediated immune response in vitro. II. The role of the thymus and thymus derived lymphocytes. Cell. Immunol. 4:39-50.

Warner, M.L., Szenberg, A. and Burnet, F.M. 1962. The immunological role of different lymphoid organs in the chicken. I. Dissociation of immunological responsiveness. Aust. J. Exp. Biol. Med. Sci. 40:373-387.

Watson, J., Mochizuki, D. and Gillis, S. 1980. T cell growth factor; Interleukin 2. Immunol. Today 1:113-117.

Weinberger, J.Z., Germain, R.N., Benacerraf, B. and Dorf, M.E. 1980. Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. V. Role of idiotypes in the suppressor pathway. J. Exp. Med. 152:161-169.

Wekerle, H., Cohen, I.R. and Feldmann, M. 1973. Lymphocyte receptors for autoantigens; autologous serum inhibits self recognition. Nature New Biol. 241:25-26.

Wekerle, H., Eshhar, Z., Lonai, P. and Feldmann, M. 1975. Thymus derived rat lymphocyte receptor for cell surface antigens is a nonserologically defined product of the major histocompatibility gene complex. Proc. Natl. Acad. Sci. USA. 72:1147-1151.

Widmer, M.B., Peck, A.B. and Bach, F.H. 1973. Genetic mapping of H-2 LD loci. Transplant. Proc. 5:1501-1505.

Willumsen, J. and Heron, I. 1974. Cell meditated lympholysis in man. A case of `nonrelevant' killing of third party persons. Tissue Antigens 4:172-177.

Wilson, D.B. 1963. The reaction of immunologically activated lymphoid cells against homologous target cells in vitro. J. Cell. Comp. Physiol. 62:273-286.

Wolstencroft, R.A. and Dumonde, D.C. 1970. In vitro studies of cell mediated immunity. I. Induction of lymphocyte transformation by a soluble `mitogenic' factor derived from the interaction of sensitized guinea-pig lymphoid cells with specific antigen. Immunology 18:599.

Yoshida, T.O. and Andersson, B. 1972. Evidence for a receptor recognizing antigen complexed immunoglobulin on the surface of activated mouse thymus lymphocytes. Scand. J. Immunol. 1:401-408.

Zar, J.H. 1974. Biostatistical Analysis, Prentice-Hall Inc., Englewood Cliffs, N.J.

Zinkernagel, R.M. and Doherty, P.C. 1974a. Restriction of in vitro T cell mediated cytotoxicity in lymphocyte choriomeningitis within a syngeniec or semi-allogeneic system. Nature 248:701-702.

Zinkernagal, R.M. and Doherty, P.C. 1974b. Immunological surveillance against altered self components by sensitized T lymphocytes in lymphocytic choriomeningitis. Nature 251:547-548.

Zinkernagel, R.M. and Doherty, P.C. 1975. H-2 compatibility requirements for T cell mediated lysis of target cells infected with lymphocytic choriomeningitis virus. Different cytotoxic T cells specificities are associated with structures coded for in the H-2K or H-2D. J. Exp. Med. 141:1427-1436.

Zinkernagel, R.M., Callahan, G.N., Althage, A., Cooper, S., Klein, P.A. and Klein, J. 1978. On the thymus in the differentiation of 'H-2 self' recognition' by T cells: Evidence for dual recognition? J.Exp. Med. 147:882-896.

END

1 1H09H84

FIN